UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

EFFECTS OF THE ADMINISTRATION OF A BOLUS OF 7,2% HYPERTONIC SALINE SOLUTION IN HORSES WITH COLIC, AS A PART OF MEDICAL TREATMENT: A PRELIMINARY STUDY.

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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Effects of the administration of a bolus of 7,2% hypertonic saline solution in horses with colic, as a part of medical treatment: a preliminary study.

Catarina Stilwell

Abstract

The present study is a preliminary study that aims to determine the existence of a beneficial effect in the administration of a bolus of 7,2% hypertonic saline solution, as a part of medical treatment, to horses suffering from gastrointestinal colic and in need of IV fluid therapy.

Eight horses were enrolled in this study and divided into two groups: LRS group that received a 2L bolus of lactated Ringer's solution and HSS group that received a 2L bolus of 7,2% hypertonic saline solution. Blood pressures and several blood parameters were collected for all horses before (T0), immediately after (T1) and 90 minutes after (T2) the administration of the fluid bolus. Between T1 and T2 all horses received approximately 10 litters of plasma - Lyte A, intravenously. No horse received oral fluids for the duration of this study.

Blood pressures increased at T1 for the HSS group while all blood parameters decreased, except for blood urea nitrogen which remained similar to T0. At T2, the blood pressures for the HSS group were lower than at T1 but higher than at T0. Lactate concentrations decreased continuously for the HSS group and increased at T1 for the LRS group.

Despite the small sample of horses used in this study, there seems to exist strong evidence of the beneficial effects of the administration of hypertonic saline solution to horses with medical colics.

Key words: hypertonic saline solution, equine colic, lactated Ringer's solution, fluid therapy

Efeitos da administração de um bólus de soro salino hipertónico a 7,2% em cavalos com colica, como parte do tratamento médico: um estudo preliminar.

Catarina Stilwell

Resumo

O presente estudo tem como objectivo determinar a existência de um efeito benéfico na administração de um bólus de soro salino hipertónico a 7,2%, como parte do tratamento médico, a cavalos com cólica gastrointestinal e com necessidade de receber fluidoterapia IV.

Foram admitidos oito cavalos neste estudo e divididos em dois grupos: grupo LRS que recebeu um bólus de 2L de lactato de Ringer e o grupo HSS que recebeu um bólus de 2L de soro salino hipertónico a 7,2%. Pressões sanguíneas e vários parâmetros sanguíneos foram avaliados em todos os cavalos antes (T0), imendiatamente depois (T1) e 90 minutos depois (T2) da administração do bólus de fluido. Entre T1 e T2 todos os cavalos receberam cerca de 10 litros de plasma - Lyte A, intravenosamente. Nenhum cavalo recebeu fluidos orais durante a duração deste estudo.

As pressões sanguíneas aumentaram em T1 no grupo HSS enquanto todos os parâmetros sanguíneos diminuíram com execepção da ureia, que permaneceu semelhante a T0. Em T2, as pressões sanguíneas para o grupo HSS eram inferiores a T1 mas superiores a T0. As concentrações de lactato diminuíram continuamente para o grupo HSS mas aumentaram em T1 para o grupo LRS.

Apesar da reduzida amostra usada neste estudo, parecem existir fortes evidências dos efeitos beneficos da administração de soro salino hipertónico a cavalos com cólicas médicas.

Palavras-chave: soro salino hipertónico, cólica equina, lactato de Ringer, fluidoterapia

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Acronyms

AG	Anion gap
ARDS	Acute respiratory distress syndrome
BUN	Blood urea nitrogen
BW/TG	Bladder width/tail girth
CaO_2	Arterial oxygen content
CI	Cardia index
CO	Cardiac output
CVP	Central venous pressure
CvO_2	Venous oxygen content
DO_2	Oxygen delivery
Hb	Hemoglobin
HSS	Hypertonic saline solution
IM	Intramuscular
IV	Intravenous
LDH	Lactate dehydrogenase
LRS	Lactated Ringer's solution
MAP	Mean arterial pressure
MCFP	Mean circulatory filling pressure
NADH	Nicotinamide adenine dinucleotide
NIBP	Noninvasive blood pressure
OER	Oxygen extraction ratio
PCV	Packed cell volume
SaO_2	Oxygen saturation
SIRS	Systemic inflammatory response syndrome
TP	Total protein
TPR	Total peripheral resistance
TS	Total solids
VO_2	Oxygen uptake
VR	Venous return
VRst	Venous resistance

Internship report

This work was performed during the 6th year of the Integrated Masters in Veterinary Medicine of the Faculty of Veterinary Medicine, University of Lisbon, under the supervision of Dr. Benjamin Buchanan and Professor Paula Tilley.

I did my internship at the Brazos Valley Equine Hospital (BVEH) in Navasota, Texas, USA, where I spent about 5 months split in two periods. For the first period I lived at the hospital from September 28 to December 18 and worked with the senior practitioners during the day and with the interns during the night as an intensive training period. For the second time, from April 4 to June 14 I did not live at the hospital but spent my days with the practitioners and interns, only driving to the hospital at night if one of the cases for my study was coming in as an emergency.

The BVEH Navasota consists of a fully equipped laboratory, a surgery room with two induction/recovery rooms, two appointment rooms with two sets of stocks each and facilities to hospitalize more than 50 animals including an ICU unit with four stalls and an isolation unit with two stalls. At the time of my internship three board-certified specialists in Surgery, Internal Medicine and Emergency/Critical Care, and Reproduction as well as two more practitioners worked at the hospital. The hospital's caseload is quite large and consists mostly of horses and ponies (95%) but also donkeys, miniature donkeys, llamas and alpacas. In 2015 the hospital attended 13,903 animals split approximately in 40% sports medicine, 20% internal medicine, 10% dentistry/wellness, 10% reproduction, 5% complimentary medicine, and 5% pre-purchase examinations.

Through my training period I perfected skills like administering medication IM, IV and orally, passing nasogastric tubes and checking for gastric reflux, placing IV catheters, performing a complete physical exam in adults and neonates, flexing limbs for lameness exams and evaluating lameness, applying bandages, suturing and caring for wounds, among others. I also got the chance of learning numerous new skills such as performing an upper airway, tracheal and gastric endoscopy, doing a bronchoalveolar lavage, flushing guttural pouches, performing a temporary tracheostomy on cadavers and assisting surgery for permanent tracheostomy (Figure 1), learning how to ultrasound lungs, abdomen, umbilicus and limbs, doing nerve and skin blocks and regional limb perfusions, placing subpalpebral lavage systems, performing abdominocentesis, measuring central venous pressure, assisting surgery and anesthesia and using several laboratory tests and machines.

Figure 1: Permanent tracheostomy performed after a temporary one had been performed in a more rostral position.

I had the opportunity of witnessing several surgeries including for colics by intestinal strangulation, impaction, torsion or obstruction by a foreign body (Figure 2), various orthopedic cases of fractures, arthrodesis, joint or tendon sheets lavages, correction of angular and flexural defects and osteochondrosis, one case of an ethmoid hematoma, one resolution of an esophageal fibrotic scar, one paranasal sinus cyst and several sinusitis from rotten teeth roots, many permanent tracheostomies due to nasopharyngeal cicatrix syndrome, various lacerations (Figure 3), several castrations, umbilical hernia resolutions and two enucleations. I also had the chance to watch Dr. Dennis Brook, from the University of Florida, performing three cataract surgeries and one iris abscess removal.

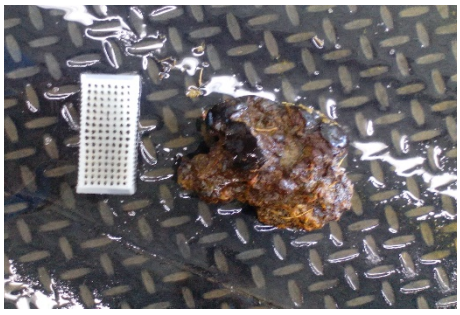


Figure 2: Calcified rope removed from the small colon of a 5yo mare with acute colic, very painful and with low response to analgesia and sedation. Nail brush for scale.

Figure 3: Two large lacerations. The horse on the left had a big sequestrum on the dorsal aspect of the cannon bone that was later removed.

In the internal medicine, neonatology and emergency/critical care area I saw several interesting cases including several infections by *Streptococcus equi equi* (Strangles), two resolutions of large colon right displacement by rolling the horse and trocharising the large colon through the abdominal wall, numerous medical resolutions of gastrointestinal colics, several cases of Equine Asthma (Figure 4), several adult horses, foals and one zebra with diarrhea (Figure 5), two cases of very severe suppurative pneumonia that ended up in one of the horses dying and the other being euthanized, two cases of dummy foals one of which suffered three fractured ribs during labor having to be submitted to surgery at 48h of life and surviving to recover completely (Figure 6), several *Rhodococcus equi* infections in foals with systemic complications on a miniature

Figure 4: Horse with Equine Asthma using an aerosol mask.

Figure 5: One year old zebra with severe diarrhea receiving IV fluids.

Figure 6: 48h old dummy foal with oxygen line and feeding tube after surgery for correction of three ribs fractured during labor.

Figure 7: The first hoof to fall from a septic two month old filly.

foal that ended up dying, one hyper-acute laminitis secondary to septicemia in a two month old filly that lost three hooves and had to be euthanized (Figure 7) and one diaphragmatic hernia from unknown cause with entrapment of the liver and spleen and splenic rupture on a 20 year old horse that was euthanized 24h after being admitted to the hospital.

I also observed the use of acupuncture to manage pain in several post-operative cases (Figure 8) and as treatment or therapeutic aid for cases of Equine Asthma, sweeney shoulder, laminitis, back and neck pain and navicular syndrome. On the reproduction area, I assisted with semen collection, natural breeding and artificial insemination, helped synchronizing oestrus and foaling mares (Figure 9) and witnessed laser cauterization of uterine cysts on four mares (Figure 10).

Figure 8: Use of acupuncture to manage pain on a 12yo mare with laminitis after colic surgery.

Figure 9: First time foaling mare and Dr. Modesto assisting the foal to stand and nurse.

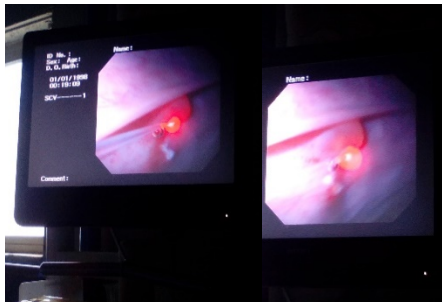


Figure 10: Laser cauterization of uterine cysts.

I thought that one surgical case was particularly interesting. A nine year old Arabian mare, seven months pregnant, was observed at the farm with acute signs of abdominal colic. The mare had had surgery for colic about a year before that and showed to be very painful. As the response to analgesia and sedation was weak and brief she was immediately refered to the hospital with indication for abdominal surgery. Surgery was conducted normally being that the cause for colic was an entrapment of the small intestine through a net in the mesentery, probably originated on the previous surgery. A resection and anastomosis was performed and the mare was up in the stall shortly after. The *in utero* foal showed good signs of vitality after surgery but progesterone was still administered orally to the mare for the time of recovery. The post-surgery antibiotic therapy was done following the hospital's protocol with penicillin and gentamicin IV as well as analgesic and anti-inflammatory therapy with flunixin meglumine IV. For four days following surgery the mare kept refluxing large amounts of fluid and feed material. By the fourth day she received a one liter bolus of 7,2% hypertonic saline solution IV on the grounds that it would decrease the swelling of the anastomosis site, improving motility and reducing the amount of reflux produced. On the fifth day the mare stopped producing reflux and was fed a liquid meal made with senior equine feed dissolved in water. For the next week the same meal was provided every six hours and alfalfa hay was introduced gradually to the mare's diet. By the end of the second week post-op the mare started showing signs of abdominal discomfort and spiked

a fever. On abdominal ultrasound the foal showed no signs of vitality and it was spontaneously aborted later that same night. Even though there were no signs of retained placenta or any further complications related to the abortion, the mare's condition kept worsening and she started refluxing again having to be taken back to surgery by the end of the third week after being admitted to the hospital. Although the anastomosis site showed no signs of complications, there were some small intestine adhesions that were considered the cause of colic and the surgery went on without complications. For the following weeks the mare recovered well from surgery but developed severe laminitis on both front feet. Besides special shoeing and medical treatment, acupuncture was also used to manage pain. At the end this mare stayed at the hospital for about six months and went home almost fully recovered.

Introduction

Colics are among the most important medical problems in equine medicine and, despite their relevance, there is still a lot to be understood about this disease (Cohen, 2002).

There is a constant search for better ways to improve recovery and survival of horses with colic and the use of hypertonic saline solution (HSS) has been a target of this quest.

It's been almost a century since the first benefits of hypertonic saline solutions were discovered but it wasn't until after 1960 that this solution's effects on hypovolemic animals started being investigated. The effects of HSS on equine colics only became a focus of investigation in the 80's when there was an increase of interest in this solution's properties.

Before the benefits of HSS in hypovolemic animals were discovered, intravascular fluid deficits had to be corrected by administrating large volumes of isotonic crystalloid fluids. The process of administering large volumes of fluids implies a longer resuscitation time and an increased risk of fluid overload, besides the fact that, in field situations, it may reveal hard to have access to such amounts of fluids. Although there are some situations where the use of hypertonic solutions is contraindicated, in hypovolemic animals this type of fluids will cause a considerable expansion of plasma volume, alongside with other effects, which will secure organ perfusion and vital functions for enough time to allow the necessary volume of replacement fluids to be administered.

Other benefits of hypertonic saline solutions, besides plasma expansion, have been studied and include the improvement of cardiac function, venodilation, immunomodulatory and anti-inflammatory properties and reduction of reperfusion injuries, among others (Schmall, 1989; Reitan, & Moore, 1998; Kien, Kreimeier & Messmer, 2002; Oliveira, Velasco, Soriano, & Friedman, 2002; Pantaleon, 2006; Strandvik, 2009; Fielding & Magdesian, 2011; Urbano et al., 2012 Chimabucuro et al., 2014;).

Although most of the existent literature about the use of hypertonic solutions in horses with colic seems to focus on surgical cases, we believe that medical cases can also benefit from the effects of this solution in a way that allows for faster recovery and lower mortality rates.

CHAPTER I – Bibliographic Review

1.1. Equine Colic

The term colic is used to describe the presence of abdominal pain, being, in reality, a generic term for a much more complex setting of problems with a variety of causes (Bentz, 2004a).

There are nearly 100 recognized causes for colics in horses and although many go by undiagnosed, especially the ones treated in the field, there seems to be a certain correlation between age, sex and breed and specific types of colic (Cohen, 2002). For example, young animals seem to be more prone to intussusceptions while strangulating lipomas are more common in older horses. Although there isn't a consistent relation between colic occurrence and gender there are some forms of colic that happen exclusively in one of the genders, such as uterine torsions in mares and scrotal herniation in stallions and geldings, and others that seem to be more frequent in some sexes despite the lack of epidemiologic studies like colonic torsions in mares after parturition (Cohen, 2002). Older horses also appear to be in higher risk of colic and of needing surgical treatment but also have better prognosis for survival (Cohen, 2002). The Arabian breed has been reported to have increased risk of colic, younger miniature horses have a bigger prevalence of fecaliths and impactions of the small colon, and Standardbreds have a higher risk of scrotal herniation (Cohen, 2002).

Some of the most common causes for equine colic not related to the GI tract, also known as "false colics", include the reproductive tract: uterine torsion, dystocia, ovulation, retained placenta, uterine hematoma or perforation and orchitis; the urinary tract: ruptured bladder, cystitis, pyelonephritis and calculi of different etiology; hepatic or splenic causes: acute hepatitis and splenic abscess; peritonitis and neoplasia (Bentz, 2004b; Mair, 2002b) .

It is important to remember that, when looking for the cause for a colic, one must not forget about other conditions that, even though are not related with abdominal pain, may mimic some clinical signs and lead to confusion about the diagnose. These disorders can be related, among other causes, with the musculoskeletal system: laminitis, rhabdomyolysis; the cardiorespiratory system: pneumonia, pleuritis; the cardiovascular system: acute hemorrhage, myocardial infarction or pericarditis; the nervous system: tetanus, botulism, hypocalcemic tetany or equine motor neuron disease; or unspecific like intoxications by Cantharidin (Bentz, 2004b; Mair, 2002b).

Predisposing factors, besides the signalment referred above, for the occurrence of gastrointestinal colics are described on Table 1.

Table 1: History findings, predisposing factors and potential mechanisms for the occurrence of colics in horses. Adapted from Blikslager and Jones (2010) and Cohen (2002).

History findings, predisposing factors and potential mechanisms for the occurrence of gastrointestinal colics.		
History finding	Predisposing factor	Potencial mechanisms
Feeding	Recent change in feeding Large infrequent meals	Alteration on fluid flux or fermentation in the large colon
	Hay with high fiber content	Obstruction of ileum by fine, fibrous hay
	Feeding round bales	Poor quality hay
	Feeding off the ground	Ingestion of sand from soil
	Bolting feed	Large boluses of feed entering esophagus and stomach
Enviroment	Excessive time in stall	Insufficient intake of roughage Insufficient exercise
	Insufficient access to water	Dehydration
	High density housing	Stress, bolting feed
Exercise	Exaustion	Dehydration Reduced GI motility
Preventive care	Insufficient dental care	Poor mastigation of feed
	Insufficient deworming	Large parasite burden
Medication	Excessive NSAIDs administration	Mucosal damage, particularly in the stomach and colon
Medical history	Colic surgery	Adhesions Anastomotic obstruction
Farm management	Bad caretaker	Lack of appropriate care of horses with previous history of colic
Weather	Warm months	Dehydration from sweating
	Cold months	Reduced water intake

Given the presented study was based solely on adult horses suffering from hypovolemia due to gastrointestinal colic, I will focus on a deeper review about gastrointestinal colics in horses and their repercussions in the body's fluid balance.

1.1.1. Hypovolemia and dehydration

One must be aware of the differences between hypovolemia and dehydration since both conditions require distinct approaches (Corley, 2008a). Even though both conditions may be present in the same horse, hypovolemia is the term used to describe the reduction of circulating blood volume whereas dehydration is the loss of interstitial fluid without decline of volemia (Corley, 2008a).

Hypovolemia can be considered absolute, where there is actual loss of intravascular volume due to diarrhea, hemorrhage or increased capillary permeability, among others; or relative, when the plasma volume remains the same but the intravenous compartment expands like in situations of systemic venodilation following SIRS or the administration of vasodilatory drugs (Magdesian, 2015b). Hypovolemia compromises organ perfusion and might result in permanent organ damage and death if not addressed correctly and in time (Magdesian, 2015b).

Hypovolemic horses that have lost 8 -10L of plasma volume, which represents about one quarter of total blood volume for a 500Kg horse, show clinical signs like slow jugular fill, tachycardia and tachypnoea, reduced urine output, cold extremities and weak pulse (Corley, 2008a; Hassel, 2015; Magdesian, 2015b). The main therapeutic goal for hypovolemia is to quickly replace blood volume, increase blood pressure and cardiac output and secure organ perfusion, which can be achieved by administering large volumes of isotonic crystalloid solutions, smaller volumes of hypertonic solutions or an association of both (Corley, 2008a; Magdesian, 2015b).

Horses that have fluid losses of more than 5% of their body weight may show dehydration signs such as tacky or dry mucous membranes, decreased skin turgor, sunken eyes and tachycardia (Corley, 2008a; Hassel, 2015; Magdesian, 2015a). Due to the body's physiologic mechanisms to maintain an appropriate circulating volume by drawing extravascular fluid into the vessels, hypovolemia due to dehydration only happens in severely dehydrated animals (Corley, 2008a). Fluid therapy in dehydrated horses is aimed at replacing interstitial fluid losses over a period of 12 to 24 hours, which should be achieved through the administration of isotonic crystalloid solutions (Corley, 2008a; Magdesian, 2015a).

1.1.2. Hemodynamic changes in colic

Total body water content of an adult horse is estimated to be about 60% to 70% of the body weight and is divided into three major spaces or compartments: intracellular (ICF), intravascular and interstitial, the last two are commonly referred as extracellular fluid compartment (ECF) (Fielding, 2015a; Seahorn & Seahorn, 2003).

Water present in the adult horse's gastrointestinal tract is thought to be approximately 6 to 10% of total body weight (Seahorn & Seahorn, 2003). The degree of fluid deficit of a horse with colic may vary from mild dehydration to hypovolemic shock (Seahorn & Seahorn, 2003).

Factors such as type of colic, duration of colic and ongoing losses should be taken into account when assessing the circulatory status of a horse with colic (Divers, 2002; Hassel, 2015). Fluid losses through sweating, increased gastric and intestinal secretions, decreased water intake and decreased intestinal absorption all contribute more or less for the onset and aggravation of fluid derangements (Divers, 2002; Hassel, 2015).

Initial losses will cause a reduction in extracellular fluid but as fluid depletion progresses, intracellular fluid may be lost as well (Divers, 2002; Hassel, 2015). Most horses suffering from colic for more than a day will show clinical signs of dehydration (Hassel, 2015). However, acute processes, such as strangulating lesions and proximal obstructions, will likely be accompanied mainly by hypovolemia secondary to a number of events such as endotoxemia, hypersecretory states, and increases in endothelial permeability (Hassel, 2015).

Loss of integrity of the gastrointestinal barrier will promote the passage of endotoxin producing Gram negative bacteria leading to endotoxemia and, more severely, to endotoxic shock (Pantaleon, 2005). Endotoxemia may originate a capillary leak syndrome which is described as the loss of fluid, with or without protein, into the interstitium due to increased endothelial permeability (Pantaleon, 2005). Endotoxemia also leads to arterial and venous dilation and, eventually, decreased cardiac output, which aggravate even further the patient's hypovolemic status (Pantaleon, 2005).

Situations of intestinal obstruction can also result in increased capillary filtration or leakage (Seahorn & Seahorn, 2003; Hassel, 2015). Intestinal obstructions result in distension of the proximal portion of intestine due to accumulation of digesta, fluid, gas and secretions. The increased intraluminal pressure compresses the veins in the intestinal wall and increases the capillary filtration rate into the interstitium. If the rate of fluid secretion is greater than the one removed by lymph flow, then edema and accumulation of fluid within the intestinal and/or gastric lumen increase (Freeman, 2002). Distension of the intestinal lumen can be such that causes necrosis of the surrounding tissues (Blikslager & Jones, 2010b).

Plasma protein losses, through diarrhea, into the intestinal lumen, in abdominal effusion, and due to increased permeability of the endothelium contribute for the loss of oncotic pressure

within the vessels and to the onset or worsening of intravascular fluid loss and tissue edema (T. Divers, 2002; Seahorn & Seahorn, 2003).

1.1.3. Fluid therapy according to type of colic

Even though there are innumerous causes of colic in horses, it is possible to divide the most common into non-strangulating mechanical obstructions, strangulating obstructions and functional obstructions (Hassel, 2015).

1.1.3.1. Non-strangulating mechanical obstructions

Non-strangulating obstructions can be partial or complete and are caused by an intraluminal obstacle to the progression of digesta without, however, causing obstruction of the blood flow (Bentz, 2004b; Blikslager & Jones, 2010b). Nonetheless, non-strangulating obstructions may result in vascular obstruction as explained on chapter 1.1.1. Some of the most common non-strangulating mechanical obstructions are originated by feed, sand or ascarids' impactions, colonic displacements, foreign body obstructions, sand impactions, enteroliths, enlargement or thickening of the intestinal wall, masses, and intestinal scarring (Bentz, 2004b; Blikslager & Jones, 2010b; Hassel, 2015).

Horses with non-strangulating obstructions normally show signs of moderate dehydration but not hypovolemia, therefore, fluid therapy for these cases aims mostly at restoring hydration and electrolytes balance using mostly isotonic crystalloid solutions (Hassel, 2015). In situations of impaction, fluid therapy may also have the goal of hydrating and dissolving the impaction, even if the horse's hemodynamic status is normal (Blikslager & Jones, 2010b; Hassel, 2015).

Colonic impactions have traditionally been treated by administering large volumes of IV fluids to achieve systemic overhydration, associated to oral administration of a laxative like mineral oil, sodium sulfate or magnesium sulfate (Bentz, 2004b; Hassel, 2015; Lopes, White, Donaldson, Crisman, & Ward, 2004), however, the amount of IV fluids administered in these situations involve some risks and haven't proved to be more effective than the oral administration of similar volumes (Hassel, 2015; Lopes, Walker, White, & Ward, 2002). Aggressive IV fluid therapy is justifiable in horses that do not tolerate enteral fluids, either due to the existence of gastric reflux previous to oral administration of fluids or caused by it, and in severe cases where enteral fluids alone are not enough (Hassel, 2015; Lopes et al., 2004).

Some colonic displacements can also be managed through IV fluid therapy (Hassel, 2015).

1.1.3.2. Strangulating obstructions

A strangulating obstruction results from the complete or partial obstruction of the intestinal lumen and corresponding blood supply and are commonly caused by intestinal volvulus, strangulating

lipomas, intussusceptions, inguinal and diaphragmatic hernias and entrapments in the epiploic foramen, gastrosplenic ligament or mesenteric rents (Bentz, 2004b; Blikslager & Jones, 2010a; Hassel, 2015).

Strangulating obstructions can be classified as hemorrhagic or ischemic. Hemorrhagic strangulating lesions happen when the intestine is twisted or compressed only to the point where veins are occluded but arteries are not, due to their more rigid wall. The affected intestine is dark and enlarged as blood flow keeps being pumped into the vessels but not out of them (Blikslager & Jones, 2010a). Ischemic strangulating lesions result from a tightly twisted intestine capable of occluding both veins and arteries and is rarer than the previous. The affected bowel is pale and of normal or thinner thickness (Blikslager & Jones, 2010a).

Both types of strangulating obstructions cause quick deterioration of the patient's condition and should be handled as surgical emergencies (Hassel, 2015).

Horses suffering from a strangulating obstruction are almost always in need for IV fluid therapy as hypovolemia, endotoxemia and systemic inflammatory response syndrome (SIRS) are frequently present (Blikslager & Jones, 2010a; Hassel, 2015). The first approach to these cases should be to restore volemia and secure organ perfusion. The administration of hypertonic saline solution associated or not to a colloid solution is often advised to quickly promote plasma expansion and improve microcirculation, organ perfusion and cardiac function besides other benefits that will be further explained on chapter 1.4. (Blikslager & Jones, 2010a; Hassel, 2015). To assure the maintenance of these effects one must provide the appropriate amount of isotonic polyionic solutions following the administration of the hypertonic solution (Hassel, 2015).

1.1.3.3 Functional obstructions

Functional obstructions are the consequence of an inflammatory condition known as duodenitis-proximal jejunitis, proximal enteritis or anterior enteritis, characterized by inflammation of the proximal small intestine and resulting in ileus (Bentz, 2004b; Hassel, 2015). Although the etiology is not yet fully understood, it is believed that *Clostridium spp., Salmonella spp.* and some mycotoxins play an important role in the development of anterior enteritis (McConnico, 2010; Hassel, 2015). The inflammatory process and reduction in motility lead to a large accumulation of fluid in the small intestine and stomach (Bentz, 2004b; McConnico, 2010; Hassel, 2015). As fluid accumulates, the stomach and small intestine become distended and painful and the horse becomes dehydrated and hypovolemic (Bentz, 2004b; Hassel, 2015). Many cases of anterior enteritis are accompanied by SIRS, which contributes even further for the depletion of the horse's hemodynamic status (Hassel, 2015). Horses with functional obstructions produce large amounts of gastric reflux that can sometimes be spontaneous (Hassel, 2015; McConnico, 2010). These horses almost always have prerenal azotemia and

electrolyte derangements (Johnston & Morris, 1987; Bentz, 2004b; McConnico, 2010; Hassel, 2015).

Aggressive IV fluid therapy should be started as quickly as possible. Hypertonic saline solution or colloids can prove useful in restoring plasma and should be followed by large volumes of isotonic polyionic crystalloid solution (McConnico, 2010). When planning fluid therapy for horses with functional obstructions it's important to have in account that these horses are in great need for fluid replacement and of expansion of the plasma volume, and that the ongoing losses from gastric reflux are very significant. However, because of the high secretion of electrolytes and protein into the intestinal lumen, horses with anterior enteritis tend to have low plasma oncotic pressure which means that, with fluid resuscitation there is also an increased risk of edema formation and further fluid secretion into the intestinal lumen. Therefore, the use of synthetic or natural colloids should be considered for horses with low plasma protein concentrations (Hassel, 2015; McConnico, 2010).

1.2. Fluid therapy

1.2.1. 0,9% Sodium chloride and Isotonic polyionic solutions

Although 0,9% Sodium chloride is usually known as isotonic or normal saline, it is actually slightly hypertonic compared to horse plasma (Magdesian, 2015a). Its composition is mildly hypernatremic and significantly hyperchloremic compared to horse plasma being, therefore, recommended for the treatment of hyponatremic and hypochloremic patients. Also, because of its high concentration in chloride and absence of a buffer component, this solution's acidifying properties can lead to a hyperchloremic metabolic acidosis (Cazzolli & Prittie, 2015) but, at the same time, make it a suitable treatment for hyperkalemic patients such as postpartum mares with a ruptured bladder (Cook & Bain, 2003). Animals being treated with 0,9% sodium chloride should be closely monitored for electrolyte disturbances since this is a solution lacking all other electrolytes in its composition.

Isotonic polyionic solutions (IPS) are considered balanced solutions and are more physiologic than isotonic saline solution since they have an electrolyte composition similar to plasma as well as a buffer component, making them a better fit for most fluid resuscitation cases (Cazzolli & Prittie, 2015). This type of fluids can be classified as maintenance or replacement solutions according to their composition (Cook & Bain, 2003; Magdesian, 2015a).

Maintenance IPS have a higher concentration of potassium and a lower concentration of sodium which allows for a long-term administration without the risk of hypernatremia or hypokalemia occurring (Cook & Bain, 2003). Maintenance fluid rate is considered to be 2,5 mL/kg/h or 60 mL/kg/d for adult horses (Cook & Bain, 2003; Magdesian, 2015a).

Replacement IPS have a relatively low potassium concentration (Table 2) making them similar to the extracellular fluid and ideal for situations where fluid resuscitation is required. The low potassium concentration of these fluids prevents the risk of iatrogenic hyperkalemia due to the high rate of which these fluids need to be administered (Cook & Bain, 2003; Magdesian, 2015b).

Table 2: Composition of different types of crystalloid solutions used in equine medicine compared to horse plasma. Adapted from Magdesian (2015b) and Cook and Bain (2003).

Type of Solution	Na+ (mEq/L)	Cl- (mEq/L)	K+ (mEq/L)	Ca+ (mg/dL)	Mg+ (mg/dL)	pH	mOsmol/L	Buffer
Horse Plasma	139	104	3,5	12,4	2,5	7,38	285	Bicarbonate
0,9% sodium chloride	154	154	0	0	0	5,0	308	None
7,2% HSS	1232	1232	0	0	0	4.0	2464	None
LRS	130	109	4	3	0	6,5	272	Lactate
Normosol – R	140	98	5	0	3	6,6	295	Acetate Gluconate
Plasma-Lyte A	140	98	5	0	3	7,4	295	Acetate Gluconate
Plasma Lyte-148	140	98	5	0	3	5.5	295	Acetate Gluconate

LRS: Lactated Ringer's solution

Normosol-R, Plasma Lyte-148 and Plasma Lyte- A are the most common replacement solutions for horses. These fluids differ only in their pH, making Plasma Lyte-A the most physiological for horses (Magdesian, 2015b). These fluids contain acetate and gluconate as buffers and therefore are advised in patients with liver dysfunctions since acetate is metabolized by muscle, liver and kidney cells and gluconate is metabolized by the big majority of body cells (Cook & Bain, 2003; Magdesian, 2015b).

Because lactate is metabolized by the liver and kidney, the presence of hepatic abnormalities may be a contraindication for the administration of Lactated Ringer's Solution or Hartman's solution (Cook & Bain, 2003; Magdesian, 2015b).

1.2.2. Risks of large volume resuscitation with crystalloids

Crystalloid fluids bear negligible oncotic pressure and they're main electrolyte is sodium. Sodium is also the main electrolyte present in the extracellular space being that about 75% of it is located in the interstitium (Cook & Bain, 2003; Cazzolli & Prittie, 2015). This fact explains why about 60 to 80% of the infused volume disseminates into the interstitial space within 30-60 minutes, making it so that considerably large amounts of these fluids must be administered before normovolemia is achieved (Cook & Bain, 2003; Magdesian, 2003; Cazzolli & Prittie, 2015).

The administration of large volumes of isotonic crystalloids may be necessary to revert hypovolemic shock and restore appropriate organ perfusion making fluid overload a common complication in human and small animal medicine (Cazzolli & Prittie, 2015). Fluid infusion should be closely monitored as a cascade of events following fluid overload will worsen the patient's prognosis (Cazzolli & Prittie, 2015). Although a significant amount of the administered fluids is believed to be excreted in urine, a high infusion rate may still result in interstitial edema formation (Cook & Bain, 2003; Fielding, 2015). As fluid load increases, the extracellular space expands, compressing and damaging the structure of the endothelial surface layer leading to capillary leak and tissue edema formation (Cazzolli & Prittie, 2015; Fielding, 2015). These types of fluids will also increase the production of inflammatory cytokines and endothelial cell activation due to their proinflammatory properties, aggravating the formation of tissue edema and hypoxia (Cazzolli & Prittie, 2015; Fielding, 2015). Other complications resulting from aggressive fluid resuscitation include dilutional coagulopathy, compartment syndromes, cellular swelling, reduced cell activity and impaired tissue healing (Cazzolli & Prittie, 2015).

In equine medicine clinical signs of fluid overload are rare in adult horses with normal renal and cardiac function. However, horses with renal failure (Corley, 2008a), increased capillary permeability and reduced oncotic pressure due to SIRS or horses with acute respiratory distress syndrome (ADRS) are more prone to getting overhydrated and developing life threatening pulmonary edema (Fielding, 2015). In these cases fluids should be administered as conservative boluses instead of continuously infused (Corley, 2008; Fielding, 2015).

Although foals barely tolerate dehydration, fluid overload in equine neonates can represent an equally threatening condition, easily leading to lung and cerebral edema and seriously compromising the cardiorespiratory, renal and hepatic systems. A close monitoring of these patients is of the utmost importance and several authors claim that, as long as renal function is secured, a small restriction of the infused volume is preferable to the risk of overhydrating and that administering fluid as boluses should only be allowed for emergency resuscitation (Corley, 2008a; Knottenbelt, Holdstock, & Madigan, 2004; Palmer, 2006; Wilkins, 2010).

Volume overload can be avoided or early detected by monitoring parameters such as central venous pressure (CVP), arterial pressure, jugular fill (Corley & Stephen, 2008; Hardy, 2010; Palmer, 2006), urine output, particularly in the neonate, urinary specific gravity, blood hematocrit and total solids (Knottenbelt et al., 2004; Palmer, 2006; Hardy, 2010). The presence of white-pink foamy nasal discharge accompanied by dyspnea is an indication of pulmonary edema, the most important sign of fluid overload, and should be assessed with furosemide given as an IV bolus and reduction or interruption of fluid administration. Some cases may require intranasal oxygen supplementation (Corley, 2008a). Changes in mental status, especially in the neonate, can also work as an indicator of the hydration status (Palmer, 2006).

1.2.3. Hypertonic Saline Solutions

A hypertonic solution is any solution with a concentration higher than its respective isotonic form. This can be achieved by using high concentrations of sodium, glucose, mannitol and other components (Kreimeier & Messmer, 2002).

Hypertonic saline solutions (HSS) have higher concentrations of sodium and chloride than isotonic saline solutions, meaning, than 0,9%. HSS are normally sold in variations of 3%, 5% or 7%, being that the ones most commonly used in equine practice are 7,2% to 7,5% (Duval, 1995; Magdesian, 2015b). HSS more concentrated than these are usually primary solutions to be used in association with other types of fluids such as dextran or hetastarch (Duval, 1995). Even though HSS are contraindicated in some situations and may involve some risks, they are generally safe and effective as well as much more affordable than colloids.

It's been 90 years since the use of hypertonic saline solutions was first described by Silbert et al (1926) as treatment for Buerger's disease or thromboangiitis obliterans (Duval, 1995). But it wasn't until 1963 that Brook's et al described the use of a hypertonic saline solution in dogs suffering from hemorrhagic shock as a way to prevent tissue damage and improve recovery. In 1980, Velasco et al increased the popularity of HSS by showing that the administration of a 4 mL/Kg bolus of 7,5% HSS to severely hemorrhagic dogs restored the blood pressure and acid-base equilibrium quickly. From the 80's forward, the number of studies concerning the efficacy of different hypertonic solutions, concentrations, doses, infusion rates and administration routes increased greatly both in human and in veterinary medicine.

In 1984, Nakayama et al used the concept of small-volume resuscitation (SVR) to describe the fast administration of a small dose of hypertonic saline solution (approx. 4 ml/kg of 2,400 mosm/L HSS) to manage hypovolemic shock as an alternative to large volumes of isotonic crystalloids (Kreimeier & Messmer, 2002).

Several mechanisms of action for the effects of hypertonic saline solutions have been suggested, studied and proven or contested. The most commonly accepted and better explained is the expansion of plasma volume. Several authors have tried to define the real increase in

plasma volume that is due to the administration of HSS, being that an increase two to five times the administered volume of HSS seems to be the most consensual values (Kien, Reitan, & Moore, 1998; Cook & Bain, 2003; Magdesian, 2015b). Fielding and Magdesian (2011) concluded that endurance horses requiring IV fluid therapy and treated with a bolus of 7,2% hypertonic saline solution had an increase in plasma volume of 29,1 ± 4% while horses treated with 0,9% isotonic saline solution had significantly smaller increase of 12 ± 14,6%, which roughly corresponds to the amount of infused fluids.

The first explanation for this expansion, to which most authors seem to agree, is a simple rise in the plasma's osmotic pressure that will cause fluid from the interstitial and/or intracellular spaces to be drawn to the intravascular compartment (Duval, 1995; Kien et al., 1998; Fielding & Magdesian, 2011). Since 96% of plasma osmolality is determined by electrolytes like sodium, chloride, bicarbonate, potassium, calcium, magnesium and phosphate, it's clear how hypertonic NaCl solutions are capable of such increases is plasma osmolality (Kreimeier & Messmer, 2002).

A study where hypertonic saline solution was administered to anesthetized rats reported not only an increase in plasma volume of four to five times the infused volume of HSS but also a reduction of 10% of the intracellular fluid volume of muscle and liver cells (Kien et al., 1998). Kien et al (1998) suggest skeletal muscle as the major mobilized fluid source for plasma expansion after HSS administration and Fielding and Magdesian (2011) state that the initial shift of fluid is from the interstitial space and only later from the intracellular space. Garrido et al (2006) consider the intracellular fluid drawn from erythrocytes and endothelial cells to be the main responsible for the immediate increase in plasma volume. Constable (2003) also includes water present in the gastrointestinal tract as a fluid source for plasma expansion.

Fettman (1985) references two authors who concluded that, following a physiological response to hemorrhagic shock that creates hyperosmolar plasma conditions, the skin and skeletal muscle represented the main sources of replacement fluid for the intravascular space. In fact, in situations of hypovolemia there is a physiological neurohumoral response that leads to changes in peripheral capillary resistance and increases in glycaemia and plasma osmolality. This mechanism promotes a fluid shift from the intracellular and interstitial compartments to the intravascular space and, at the same time, reduces the intravascular space relatively to blood volume (Fettman, 1985; Schmall, 1989). However, this physiologic response is not enough to endure a severe hypovolemic state (Schmall, 1989), so hypertonic solutions are considered to synergize with this intrinsic mechanism in order to obtain a more effective result (Fettman, 1985).

Another possible explanation is that there is a considerable thirst response triggered by the increase in plasma's sodium concentration meaning that, in cases where the patient is allowed to drink, the water intake will contribute for the plasma expansion (Fielding & Magdesian, 2011).

Hypertonic saline solutions also appear to reduce swelling of erythrocytes (Oliveira, Velasco, Soriano, & Friedman, 2002; Magdesian, 2015b) and capillary endothelial cells, often seen during hypovolemic and hemorrhagic shock and systemic inflammatory response syndrome (Kien et al., 1998; Oliveira et al., 2002; Corley, 2008a). Reducing erythrocyte swelling associated to the plasma expansion will reduce blood viscosity and improve microhemodynamics (Pantaleon, 2005). Endothelial swelling causes the capillary lumen to reduce, increasing capillary resistance and affecting the involved microcirculation, ultimately leading to tissue ischemia. Endothelial swelling also restricts the passage of large white blood cells across the vessel's walls (Kien et al., 1998). A study where rabbits were subjected to hemorrhagic shock observed that, within one minute after resuscitation with HSS, the blood volume was similar to values previous to hemorrhage and that endothelial swelling had reduced by 20% (Kien et al., 1998).

Adding to the plasma expansion effects, hypertonic saline solutions also carry vasodilator properties, mainly on peripheral vasculature. This effect is considered to be the cause for some cases of short-lasting hypotension immediately after infusion (Kien et al., 1998; Kreimeier & Messmer, 2002; Oliveira et al., 2002). HSS infusion causes the release of vasodilating substances such as prostacyclin and increases the 6-keto-prostaglandin $F_{1\alpha}$: thromboxane B_2 ratio (Oliveira et al., 2002). Prostacycline is a vasodilator and inhibitor of platelet adhesion and aggregation, released by endothelial cells (Frandson, Wike, & Fails, 2009). 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 are stable hydrolysis products of prostacyclin and thromboxane A_2, respectively. As said before, prostacyclin is a vasodilator and inhibitor of platelet aggregation while thromboxane A_2 is a vasoconstrictor and an inducer of platelet aggregation, therefore an increase in their ration will favor the prostacyclin's effects (Saldeen & Saldeen, 1983).

Kien et al (1998) reported a severe decrease in mean arterial pressure 45 seconds after the beginning of the administration of hypertonic saline solution at a rate of 2 mL/Kg/min and 3 mL/Kg/min but not with rates of and under 1 mL/Kg/min. At five minutes post-infusion the mean arterial pressure had risen to values similar to baseline for all groups (0,5, 1, 2 and 3 mL/Kg/min), suggesting that the initial hypotension was due to peripheral vasodilation leading to a reduction in peripheral resistance with distribution of the total plasma volume. The authors do not state the solution's concentration or the species of the animals used. Kien et al (1998) also showed that the hypotensive effect of HSS in dogs treated with endothelium-derive relaxing factor was significantly smaller when compared to dogs treated with autonomic nervous system blocking drugs and to not treated dogs, indicating the involvement of mechanisms directly related to the endothelium. In a study using a model for hemorrhagic shock in foals, the

administration of 32 mL/Kg isotonic saline solution or 4mL/Kg of 7% NaCl in 6% Dextran 70 produced the same significant increases in mean arterial pressure and cardiac output but the reduction in total peripheral resistance was not observed with the administration of 0,9% NaCl (Schmall, 1989). The vasodilator properties of HSS have also been proven to exist in salt-free hypertonic solutions suggesting this effect may be due to the hyperosmolar effect rather than to the presence of a salt (Kien et al., 1998).

Most of the existing literature agrees that hypertonic saline solutions increase cardiac contractility, cardiac output, stroke volume, and improve arterial blood pressures (Schmall, 1989; Schmall, Muir, & Robertson, 1990; Pantaleon, 2005; Taylor & Clark, 2007; Robertson, 2010; Theobaldo, Barbeiro, Barbeiro, Petroni, & Soriano, 2012; Magdesian, 2015b). Some authors discuss if the increase in cardiac contractility caused by HSS administration should be considered a direct inotropic effect (Kien et al., 1998; Magdesian, 2015b) as the hyperosmolality of the extracellular compartment and subsequent reduction in intracellular water may promote the release of calcium ions from the sarcoplasmic reticulum and t-tubules, resulting in higher concentrations of intracellular calcium and favoring the cardiac muscle fibers contractility (Kien et al., 1998; Pantaleon, 2005). On the other hand, Constable (2003) considers HSS to actually have a negative inotropic effect caused by the exchange of extracellular sodium for intracellular calcium promoted by the high extracellular sodium concentration, resulting, therefore, in lower intramyocyte calcium availability. This contradiction can be explained by some findings reporting that moderate degrees of hyperosmolarity will increase cardiac contractile forces whereas the opposite happens under severe hyperosmolarity conditions (Oliveira et al., 2002). Some authors seem to agree that HSS will have a vasodilator effect on the coronary arteries, improving blood supply to the myocardium (Fettman, 1985; Kien et al., 1998) while others consider the improvements in cardiac performance to be independent of the increased coronary blood flow (Oliveira et al., 2002).

There is still some discussion about the role of lung innervation on the action mechanisms of hypertonic saline solutions. It has been suggested that the high concentration of sodium stimulates pulmonary receptors, sending an impulse to the brain through the vagal nerve and resulting in a shift of blood flow from the skin and muscle to the vital organs. This was called the pulmonary arc reflex (Duval, 1995). Initial studies showed that HSS infusions to the aorta or pulmonary vein of hemorrhagic dogs, meaning post-pulmonary circulation, did not produce the same resuscitation effects as the intravenous or pre-pulmonary infusions (Schmall, 1989; Oliveira et al., 2002). On the other hand, a study conducted with sheep showed no differences in the results obtained with peripheral or central administration of HSS (Oliveira et al., 2002). In the same way some studies showed the need for the innervation to the lung to be intact for HSS to produce a systemic result (Schmall, 1989). However, other authors showed that similar effects of

resuscitation with hypertonic saline are obtained in hypovolemic and hemorrhagic dogs with innervated and denervated lungs (Duval, 1995; Oliveira et al., 2002). The vagal nerve is known to have an important function on the restoration of hemodynamics in hypovolemia situations and several authors showed that animals with vagal blockade and vagotomy produced a reduced response or even no response to the administrations of HSS (Fettman, 1985; Schmall, 1989; Duval, 1995; Oliveira et al., 2002;). The number of contradicting literature leaves several unanswered questions and creates doubt around the real involvement of this reflex on hypertonic saline solution therapy.

Hypertonic saline solutions have also been showed to have immunomodulatory and anti-inflammatory effects (Theobaldo et al., 2012; Chimabucuro et al., 2014; Magdesian, 2015b). Chimabucuro et al. (2014) studied the effects of 7,5% HSS (4 mL/Kg) and 0,9% normal saline (32 mL/Kg) in gut perfusion after ischemia by occluding the superior mesenteric artery (SMA) in rats and concluded that the immunologic effects of the administration of a small dose of HSS are similar to the ones obtained with a large dose of normal saline without the increased risk of edema production. The animals used were distributed in four groups: subjected to SMA occlusion treated with HSS, subjected to SMA occlusion treated with normal saline, subjected to SMA occlusion but not treated and animals not subjected to SMA occlusion and not treated with any type of fluid. Inflammatory and oxidative stress markers were collected for a six hour times after gut reperfusion. It is important to notice that the animals not subjected to SMA occlusion were still exposed to general anesthesia, abdominal wall incision, gut manipulation and SMA handling, having, therefore, relatively increased levels of inflammatory and oxidative stress markers. The authors noticed that both groups receiving crystalloids had significant decreases in oxidative stress markers and an improved inflammatory response when compared to the not treated group. Furthermore, the hypertonic saline solution group had higher plasma levels of interleukin 6 and 10, which are pro-inflammatory and anti-inflammatory respectively, but the levels of these interleukins in these animal's tissues were very similar to the group that was not subjected to gut ischemia. Even though the levels of pro-inflammatory cytokines are not completely annulled by HSS, authors agree that the production of IL-6 will regulate the synthesis of IL-10 and an adequate ratio between anti and pro-inflammatory cytokines is crucial for a positive outcome (Pantaleon, 2005; Chimabucuro et al., 2014). Several other studies have shown the increases in anti-inflammatory cytokines caused by hypertonic saline solution (Pantaleon, 2005; Theobaldo et al., 2012; Magdesian, 2015b).

The mechanisms for neutrophil activation, migration and infiltration are well known to play a major role in the inflammatory response to sepsis or ischemia and have been associated with damage to the endothelium, lung, intestines and other tissues, capillary leakage and tissue

edema, aggravating even further the tissue hypoxia (Garrido, Cruz, Poli de Figueiredo, & Rocha e Silva, 2006; Kreimeier & Messmer, 2002; Pantaleon, 2005; Magdesian, 2015b).

Neutrophil infiltration was reduced on the HSS and normal saline treatment groups to levels similar to the group free of gut ischemia in the study by Chimabucuru et al. (2014). The administration of hypertonic saline solution in experimental hemorrhagic shock showed to reduce neutrophil activation resulting in reduced lung injury (Corley, 2008a). Elevated levels of sodium reduce the neutrophil exaggerated response (Pantaleon, 2005). Moreover HSS reduces neutrophil accumulation in the lung, neutrophil - endothelial adhesion and neutrophil degranulation decreasing end organ injury (Pantaleon, 2005).

Other benefits of hypertonic saline solutions may include the indirect release of vasopressin and activation of aquaporin channels (Fielding & Magdesian, 2011). Vasopressin is released in both plasma hyperosmolality and hypovolemia situations being that the stimulation of volume receptors predominates over the osmoreceptors. In this way hypertonic solutions will not inhibit this hormone's secretion through their hyperosmolarity (Fettman, 1985). In hypovolemic animals, the increases in vasopressin will prevent water diuresis in order to maintain an adequate plasma volume while the activated aquaporin channels will facilitate the water to flow out of the cell (Magdesian, 2015b).

There are many conditions where the use of HSS may be indicated, either alone or combined with colloid agents. Severe burns, traumatic brain injury, lung injury, before general anesthesia and as resuscitation during circulatory and endotoxic shock are some of the most common situations that justify the use of this type of fluids (Duval, 1995; Kien et al., 1998; Garrido et al., 2006; Magdesian, 2015b).

Hypertonic saline has been used for some time to help balance fluid and electrolyte's losses in severely burnt patients and to reduce intracranial pressure in traumatic brain injury, many times as pre-hospital treatments (Fettman, 1985; Kien et al., 1998; Magdesian, 2015b).

The expansion in plasma volume and improved cardiac output promote the redistribution of blood flow from peripheral beds to vital organs and the reestablishment of microhemodynamics, reducing tissue lesions due to ischemia or inflammatory processes. There seems to be a bigger increase in blood flow to organs like heart, kidney, liver and intestines after treatment with hypertonic solutions when compared to isotonic solutions (Schmall, 1989; Kien et al., 1998).

The improved kidney perfusion and higher plasma sodium concentration after HSS infusion lead to a shorter time to urination, increased urinary frequency and more diluted urine, compared to treatment with isotonic crystalloids, which is believed to act as a protective effect against renal failure associated with hypoperfusion (Fielding & Magdesian, 2011). With the normalization of renal function, HSS is also believed to restore the renal interstitium osmolality and the countercurrent mechanisms by providing the needed electrolytes (Fettman, 1985). This effect

will help avoid medullary washout (Fettman, 1985). Medullary washout describes the loss of the medullar interstitium osmolality gradient, consequent inability to concentrate urine after restoration of renal blood flow and increased diuresis following high-volume fluid administration (Schott II, 2010; Magdesian, 2015b).

Treatment with hypertonic saline is contraindicated in the presence of hypernatremia, hyperchloremia or hyperosmolarity as these are already intrinsic risks to the administration of HSS (Fettman, 1985; Kien et al., 1998; Magdesian, 2015b).

Due to its low pH and absence of a buffer component, some clinicians worry about a possible iatrogenic acidosis caused by the administration of hypertonic saline. But according to Constable (2003) the decrease in pH caused by HSS in large animals is less than 0.08 units and it lasts little time making this a safe solution in terms of acid-base balance.

While treatment with hypertonic saline solution is indicated in some cases of hemorrhagic shock, the presence of uncontrolled hemorrhage excludes the possibility of using HSS as these solutions are reported to increase bleeding and reduce clot stabilization (Kien et al., 1998; Kreimeier & Messmer, 2002; Magdesian, 2015b).

Even though the benefits of hypertonic solutions are numerous one should have in mind that the effects of hypertonic saline solutions are of short duration and work at the expenses of the body's fluid reserves. There's some concern about giving hypertonic solutions to dehydrated patients since these may have intracellular fluid deficits and may not be able to compensate the hyperosmolar conditions. In such situations Magdesian (2015b) advises the administration of isotonic crystalloids prior or simultaneously to the HSS infusion. In patients with mild or no signs of dehydration, the administration of hypertonic solutions should be followed by adequate amounts of isotonic or hypotonic IV fluids or the patient should be allowed to drink *ad libitum* (Magdesian, 2015b).

1.2.4. Crystalloids Vs Colloids

Colloids are solutions containing large molecular weight molecules and can be divided in natural: plasma (the most used in equine medicine), whole blood and albumin; or synthetic: hydroxyethyl starch solutions (the most used in equine medicine), dextran, gelatin and polymerized hemoglobin (Magdesian, 2015b). There is an ongoing debate in the medical field about the fluid choice for resuscitation purposes in both human and veterinary medicine (Pantaleon, 2005; Chan, 2008; Magdesian, 2015b; Cazzolli & Prittie, 2015). Both crystalloids and colloids present advantages and disadvantages in their use and numerous studies have been conducted to clarify which one overcomes the other with no obvious conclusions in either human or veterinary medicine (Chan, 2008; Magdesian, 2015b). Due to the elevated price of colloids and lack of obvious benefits over crystalloids, the last ones are usually preferred over the first (Magdesian, 2015b). Although colloid solutions carry increased risks of anaphylactic

shock, coagulopathies and acute renal failure, (Chan, 2008; Cazzolli & Prittie, 2015; Magdesian, 2015b) they also provide a bigger oncotic pressure to plasma, having longer lasting effects on plasma expansion than crystalloids (Magdesian, 2003; Chan, 2008). Due to the dilutional effect of isotonic crystalloids that leads to loss of intravascular oncotic pressure, the use of colloids is advised in case of augmented capillary permeability and risk of edema formation (Cook & Bain, 2003; Magdesian, 2003; Pantaleon, 2005). However, the clinician should use colloids conservatively in patients with SIRS or severe trauma whose capillary permeability may be such that there is extravasation of colloids to the interstitium, potentiating interstitial edema formation (Magdesian, 2015b)

Renal failure has not yet been reported in horses receiving hydroxyethyl starch, commonly known as hetastarch, and to the moment there are no studies relating increased mortality with colloid treatment in equine medicine. However, a recent meta-analysis of 38 clinical trials in humans, gathering more than 10.000 patients, questioned the safety of this solution. The doubt about hetastarch's safety was further aggravated when hydroxyethyl starch was in fact correlated with increased mortality and renal failure after seven trials stating the opposite were removed from this meta-analysis due to "retraction of the original studies because of scientific misconduct on the part of the lead author" (Magdesian, 2015b).

The use of crystalloids associated with colloids has proved to be useful in multiple situations (Pantaleon, 2005). A study conducted in an infant animal model of hypovolemic shock concluded that the effects of resuscitation with 3% hypertonic saline solution or with a combination of 3% hypertonic saline colloid and 5% albumin resulted in similar hemodynamic improvements with longer lasting effects for the hypertonic saline colloid (Urbano et al., 2012). The association of colloids and hypertonic crystalloids produces a synergetic effect as colloids maintain appropriate plasma oncotic pressures while hypertonic crystalloids draw water into the vessels, conserving the plasma expansion effect for longer (Pantaleon, 2005). Adding colloids to crystalloid solutions will also reduce the risk of edema formation whereas the crystalloids may exert some protective effect against kidney damage and replace interstitial fluid losses (Magdesian, 2003, 2015b).

1.3. Central Venous Pressure

Measuring the central venous pressure is a simple, minimally invasive and useful method of assessing a patient's circulatory status and cardiac function, especially when it comes to critical care, as well as a helpful way of deciding about and monitoring the response to treatment.

In simple terms, the CVP is the pressure exerted by the blood inside the thoracic cranial vena cava and it reflects the relation between volemia, venomotor tone and cardiac function, or the blood volume of the venous compartment, the volume capacity of the vessel and the diastolic pressure of the right ventricle (Magdesian, 2004).

In the human body approximately 70% of total blood volume is contained in the venous system while only 18% is in the arterial system and the remaining in terminal arteries and arterioles (Gelman, 2008). In horses the distribution is about 60% in the venous system, 15% in arterial system and the remaining in terminal circulatory vessels (Poole & Erickson, 2008). Due to their high capacitance and high compliance, veins serve as the body's main blood reservoir and are responsible for maintaining filling pressure in the right heart trough minimal changes in transmural pressure, making them easily adaptable to changes in blood volume (Gelman, 2008).
To be clear how the CVP influences the cardiac output (CO) and circulatory status, concepts such as venous resistance (VRst), venous return (VR) and mean circulatory filling pressure (MCFP) must be understood first.
Venous resistance is simply the force that opposes blood flow when going through the systemic venous circulation and it is dependent of the length and diameter of the vessel (Gelman, 2008; Frandson, Wilke, & Fails, 2009; Bressack & Raffin, 2016;).
Gelman, 2008, describes the mean circulatory filling pressure as the blood pressure inside any vessel of the circulatory system in case the heart stopped beating for a small fraction of time and, therefore, no blood would be flowing from or to the heart and the pressure inside all vessels would be the same.
Venous return is a simple concept used to describe the amount of blood that reaches the right ventricle during diastole. According to Starling's law of the heart, the larger the venous return the larger the stroke volume ejected during the systole, meaning, the bigger is the cardiac output considering the same heart rate (Elliott & Bowen, 2010).
The venous return can be considered as the difference between the mean circulatory filling pressure and central venous pressure divided by the venous resistance (Gelman, 2008):

VR = (MCFP-CVP) / VRst

Although, in theory, the venous return can be influenced by the venous resistance, in physiologic conditions this parameter doesn't change much and is relatively small making it so that the CO is mainly influenced by the VR and the VR can be increased only by increasing the MCFP or decreasing the CVP (Gelman, 2008).
Although the previous statement seems contradictory since it's known that an increase in CVP will improve the CO in a normal heart, the fact is that, upon administration of fluids or venoconstrictors, the mean circulatory filling pressure will, too, suffer an increase equal or bigger than the central venous pressure and, therefore, the total VR will rise as well (Gelman, 2008).
The central venous pressure measurement is traditionally made inside the thoracic cranial vena cava although other possible locations such as the jugular vein, pulmonary artery and right atrium have also been reported (Wilsterman, Hackett, Rao, & Hackett, 2009).

Different procedures for measuring CVP have been described. Commercial catheters designed especially for the measurement of central venous pressure are available to sizes up to 90 cm of length but Fielding, Balaam, and Sprayberry (2004) demonstrated a new alternative for adult horses using a sterile 55 cm polyethylene or polypropylene tubing filled with heparinized fluid passed through a regular 10 or 14 gauge venous catheter. For average-sized neonatal foals a 20-30 cm jugular catheter is usually suitable (Magdesian, 2004). The catheter is then attached to a measuring device like a water manometer or to an electronic pressure transducer connected to a monitor. Attention should be payed to air within the lines or manometer and eventual catheter obstructions or kinking since these will created artificially high values (Magdesian, 2004).

While the water manometer is more portable and fairly inexpensive compared to the electric pressure transducer and monitor, it is less precise and will only allow for intermittent readings whereas the second will allow for more accurate values and a continuous monitoring (Tennent-Brown, 2015). Measurements made in mmHg can be easily converted to cmH_2O by multiplying the value by 1,36.

Ideally, the CVP measurements are referenced to the right atrium and the zero mark on the water manometer or pressure transducer should be leveled to its height. Externally in a standing horse, this corresponds to the point of the shoulder and in a recumbent horse it corresponds to the tip of the manubrium. It is important that this mark remains the same throughout different measurements so a clipped line or other kind of mark on the horse's shoulder or taping the measuring devise to a height-fixed object are practical solutions (Figure 11) (Tennent-Brown, 2015).

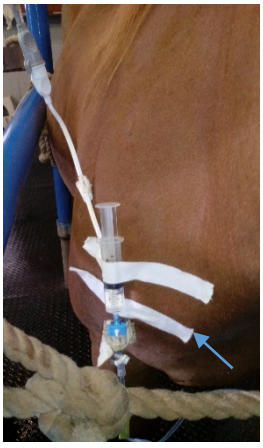
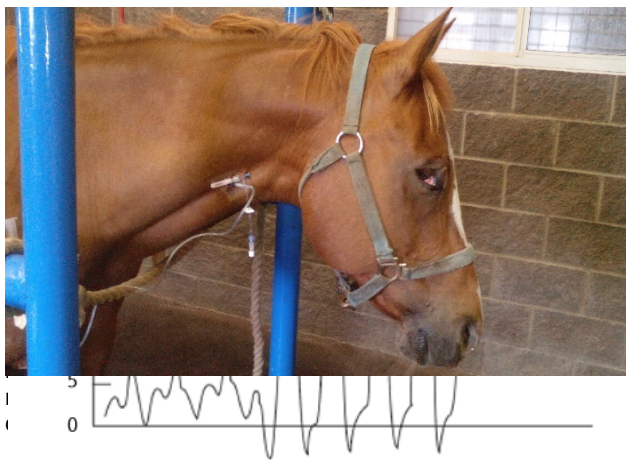


Figure 11: To avoid clipping a line in the horse's shoulder a strip of white tape (arrow) marks the "zero" for the measuring devise. This technique only works if the skin is dry and for a short period of time.

Head should be kept in a neutral position, ideally with the tip of the nose at shoulder point height, and stay constant in between measurements. Efforts should be made to restrain the horse as little as possible and nose twitches or other type of containing instruments should be avoided (Figure 12) (Norton et al., 2011; Tennent-Brown, 2015).

Large oscillations probably mean the catheter is inserted in the right ventricle or pulmonary artery in which case it should be pulled back until the oscillations are small and only due to respiration (Magdesian, 2004). In case the catheter is connected to a pressure transducer and monitor, the waveforms and pressure values can provide



information about the location of the catheter tip (Figure 13) (Tennent-Brown, 2015). The correct positioning of the catheter can also be confirmed by echocardiography (Wilsterman et al., 2009) or, in case of neonatal foals, by portable thoracic radiographs (Tennent-Brown, 2015).

It is expected for the CVP reading to slightly decrease during inspiration and to increase during expiration as intrathoracic pressure falls and rises, respectively (Cook & Bain, 2003). This oscillation is a helpful way to confirm that the catheter tip is, in fact, intrathoracic. Higher CVP values are also expected in recumbent horses during anesthesia, specially lateral recumbency (Klein & Sherman, 1977) and during positive pressure ventilation (Gelman, 2008), however, in this case, the higher values will be reached during inspiration instead of expiration (Magdesian, 2004). In any case, CVP readings should be done at the end of expiration (Magdesian, 2004).

Figure 13: Different waveforms and pressure values shown on the monitor of the pressure transducer according to the location of the CVP catheter tip (Tennent-Brown, 2015).

Sedation is sometimes required to properly handle and examine a horse. In these cases xylazine is the advised drug since it seems to have minimal influence on CVP, while acepromazine may decrease its readings (Klein and Sherman 1977; Fielding, Balaam, and Sprayberry 2004).

Measuring CVP is indicated in the assessment of patients presenting with hypovolemia, right-side heart failure and hypooncotic states, and to monitor the response to fluid therapy, patients in risk of edema formation and patients in acute stages of altered cardiac contractility (Magdesian, 2004).

Numerous studies have been carried out to establish the normal CVP values for adult horses, being that an interval ranging from 7,5 ± 0,9 to 12,0 ± 6,0 cmH_2O (Magdesian, 2004) is considered normal. On the other hand, a study done to evaluate the repeatability, reproducibility, and effect of head position on CVP measurements in standing healthy adult horses had mean CVP values of 9,4 ± 3,6 cmH_2O and 9,56 ± 4,2 cmH_2O on two different experiments (Norton et al., 2011). Another study meant to analyze the effects of hypohydration on CVP and splenic volume in healthy adult horses obtained basal CVP values ranging from 6,0 to 20,7 cmH_2O, with a mean of 11,6 cmH_2O (Nolen-Waltson et al., 2011). These results suggest that CVP values outside the expected interval may still be considered normal. Values for neonatal foals on their first 2 weeks of live range from 2,8 to 12,0 cmH_2O (Magdesian, 2004). Consequently, much more information can be obtained by relating the patient's CVP values to their clinical presentation and by evaluating each individual's CVP variation along time, either by serial or continuous measurements, instead of relying on a single measurement (Magdesian, 2004; Tennent-Brown, 2015).

A significant number of factors can affect the values of CVP readings. Some are simply physiological while others may indicate more serious events and be a reason for concerning

Norton et al., 2011, proved that head height in adult horses had a significant effect on CVP, being that the elevated head position would decrease the CVP in 2,0 ± 6,5 cmH2O and the lowered head position would increase it in 3,7 ± 5,5 cmH2O.

Although normal CVP values do not necessarily indicate euvolemia, low or negative values are most likely an indication of hypovolemia or increased vasodilation (Magdesian, 2004). When monitoring fluid therapy, the absence of an increase in CVP readings after a fluid bolus may be a sign that the hypovolemia still stands whereas an increase bigger and faster than expected is most likely a sign of fluid overload (Magdesian, 2004) or cardiac failure (Gelman, 2008). On the other hand, fluid overload may be compensated by accumulating blood in the splanchnic circulation without altering CVP values (Gelman, 2008).

Increases in CVP can also be caused by pericardia/pleural infusion, pneumothorax, pulmonary hypertension, venoconstriction or increased intrathoracic or intraabdominal pressure (Gelman, 2008; Magdesian, 2004).

One of the first mechanisms the body activates when trying to cope with hypovolemia is to shift the blood located on the splanchnic circulation onto the main venous circulation, only upon the exhaustion of this blood reserve the central venous pressure will start dropping. In humans, a clearly low CVP may represent a blood volume loss greater than 10-12% (Gelman, 2008).

In contrast, in cases of mild hypovolemia, the CVP can also drop in an attempt to decrease the MCFP-CVP gradient described above and consequently increase the venous return and cardiac output (Gelman, 2008).

An experimental model of acute blood lost showed that the decrease in CVP was noted significantly prior to the increase in heart rate, which stands once more for the importance of this tool in the early assessment of the patient's circulatory status (Magdesian, Fielding, Rhodes, & Ruby, 2006).

Situations of venodilation or blood sequestration in the splanchnic circulation also cause the CVP to decline (Gelman, 2008).

It is important to remember the innumerous coping mechanisms of the circulatory system and how an appropriate CVP is important for the cardiovascular function. The body puts all efforts in maintaining homeostasis which may lead to the presence of normal CVP values even in situations of serious hemodynamic derangements, making so that some authors only consider extreme CVP values to be noteworthy and affirm that it is impossible to build a correlation between CVP and circulating blood volume (Gelman, 2008).

1.4. Noninvasive arterial blood pressure

Even though blood flow is the actual factor that limits tissue perfusion, its direct measurement is not practicable in the day to day practice. Therefore, arterial blood pressure is the best way to

estimate blood flow and, as long as the vessel's compliance and resistance are normal, the correlation between both is generally good (Magdesian, 2004; Tennent-Brown, 2015).

Arterial blood pressure is simply described as the product of cardiac output and the systemic vascular resistance or total peripheral vascular resistance (TPR) and is usually represented as mean arterial pressure (MAP), although it is also possible to obtain systolic and diastolic values (Frandson, Wilke, and Fails 2009; Magdesian 2004): MAP = CO x TPR.

In opposite to veins, arteries have very low compliance and function as a reservoir of blood under pressure, being that arterioles contribute the most for the systemic vascular resistance. This way, arterial blood pressure is the balance between the force pushing blood into the arteries, this is, the systole, and the force that opposes the blood from exiting the arteries, meaning, the resistance created by the arterioles (Frandson, Wilke, et al., 2009).

Knowing that blood pressure is fundamental for the appropriate perfusion of tissues, the body holds innumerous mechanisms for the regulation of blood volume and arterial blood pressure. The kidneys are the organs whose function have a bigger impact in the regulation of the circulating blood volume by the activation of the renin-angiotensin-aldosterone system. This system promotes vasoconstriction and reduces urinary excretion of water and sodium chloride resulting in an increase in arterial pressure and blood volume (Frandson, Wilke, et al., 2009).

Other mechanism capable of regulating blood pressure and volume include the arterial baroreceptors reflexes, responsible for changes in heart rate, cardiac contractility, arteriolar constriction and venoconstriction; epinephrine and norepinephrine upsurges under stressful situations, leading to increases in heart rates and constriction of smooth vascular muscle; atrial natriuretic peptides that increase the excretion of sodium chloride and water by the kidneys and promote the relaxation of the arterioles in response to increases in atrial filling pressures; and endothelin and nitric oxide released by endothelial cells, with arteriolar vasoconstrictive and local vasodilating properties respectively (Frandson, Wilke, et al., 2009).

Several techniques are known and used to measure arterial blood pressure and can be divided in either invasive or direct and noninvasive or indirect methods.

Direct measurements are normally performed by catheterization of the transverse facial artery in adult horses or the great metatarsal artery in foals (Magdesian, 2004). Other locations for adult horses catheterization include the facial artery and the dorsal metatarsal artery (Taylor & Clark, 2007) and for foals are the radial and caudal auricular arteries (Magdesian, 2004). The catheters are connected to an electronic pressure transducer with monitor or to an aneroid manometer leveled with the sternum. Direct blood pressure requires more technique, especially in situations of hypotension and can result in complications such as hematomas and septic or thrombotic complications, it can also be harder to maintain the catheter in place in

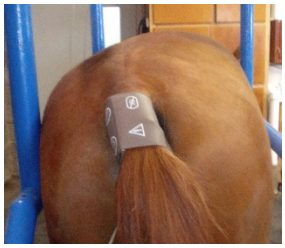


Figure 14: Inflatable cuff placement for NIBP measurements on the coccygeal artery of a mare.

an active animal (Magdesian, 2004; Taylor & Clark, 2007; Tennent-Brown, 2015). On the other hand, this technique allows for continuous monitoring and has an improved accuracy compared to the noninvasive method, especially in severe low-stroke volume or vasoconstrictive states, making it more appropriate for monitoring critically-ill patients (Fielding and Magdesian 2015; Magdesian 2004).

Indirect or noninvasive blood pressure (NIBP) can be measured through different methods being that the most commonly used in equine medicine are the oscillometric sphygmomanometry and the Doppler methods (Robertson, 2010). The oscillometric sphygmomanometry method records systolic, diastolic and mean arterial pressures through an inflatable cuff, usually placed around the coccygeal artery on the base of the tail (Figure 14) or around the dorsal metatarsal artery on the metatarsus (Hardy, 2010; Magdesian, 2004). As the cuff deflates, an oscillometric automated sphygmomanometric monitor records the changes in oscillation generated by the changes in blood flow (Corley, 2008b; Hardy, 2010). These oscillations start when the cuff's pressure reaches systolic pressure, at mean arterial pressure the oscillations are maximal and then reduce until they disappear at diastolic pressure (Hardy, 2010). A great number of oscillometric monitors is fabricated for human use which can represent a problem when measuring over the horse's slower heart rates (Corley, 2008b; Robertson, 2010). Therefore, if the heart rate displayed on the monitor does not match the horse's heart rate, the readings should be discarded (Corley, 2008b; Magdesian, 2004).

The Doppler method uses a piezoelectric probe that detects the blood flow, amplifies it and converts it in an audible signal (Hardy, 2010; Robertson, 2010). The probe must be placed over the coccygeal artery, just distally to an inflated cuff that's occluding the artery. The cuff must be attached to a sphygmomanometer. As the cuff deflates the first sounds corresponds to the systolic blood pressure (Robertson, 2010). Doppler units that detect arterial wall motion (instead of blood flow) are also able of measuring the diastolic pressure (Magdesian, 2004). This method is not accurate at measuring mean arterial pressures and it leads to considerable errors in dorsally recumbent horses (Hardy, 2010; Robertson, 2010).

The actual part of the cuff that is inflated is called the bladder (Figure 15) and it is important to have in account the bladder width to tail girth ratio (BW/TG) when aiming for the most accurate measurements (Corley, 2008b; Parry, McCarthy, & Anderson, 1984). In cases where the bladder is too wide, the readings will be lower than reality whereas if the bladder is too narrow the readings will be falsely high (Corley, 2008b; Magdesian, 2004). Parry, McCarthy, and Anderson, (1982) showed that the BW/TG ratio for optimal accuracy of systolic blood pressure should be of 0,34 while for diastolic blood pressure it should be of 0,98, in adult horses. For an accurate measurement of MAP, Magdesian

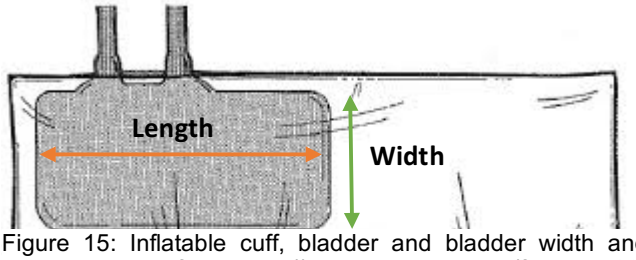


Figure 15: Inflatable cuff, bladder and bladder width and length. Adapted form: http://drrajivdesaimd.com/?paged=20

(2004) affirms that the BW/TG ratio should be of 0,2 to 0,25 for the tail and 0,4 to 0,5 for the limb. The bladder's length will also play a role in arterial blood pressure measurements and it should be of about 80% of the circumference of tail or limb (Magdesian, 2004). Cuffs that are too loose or too tight around the appendage will also provide falsely low or high measurements, respectively (Magdesian, 2004).

Arterial blood pressure from the coccygeal artery can be corrected to the level of the heart or stated as uncorrected "coccygeal values". In standing horses, coccygeal blood pressure tends to be 15-20 mmHg lower than the corrected value since the artery is elevated is relation to the heart. The opposite happens in horses in dorsal recumbency. The most important factor to have in consideration is the consistency among measurements, more than their correction for height or not (Magdesian, 2004).

When measuring NIBP it's important to follow some recommendations for a better accuracy of the technique and higher reliability and consistency of the results obtained. The horse's head should be kept in a neutral position like the one explained for CVP measurements (Magdesian, 2004). Stress and excitement ought to be reduced as much as possible and the animal should be allowed to get used to the inflation and deflation of the cuff before one begins to record the given values as some horses might contract their coccygeal muscles for the first couple of cycles (Magdesian, 2004; Parry et al., 1984).

A survey of resting blood pressure in clinically normal horses found significant differences in diastolic and systolic blood pressure between horses considered nervous/timid, normal/quiet and very quiet (Parry et al., 1984). The same survey also reported significantly higher values for Thoroughbreds when compared to Standardbreds and hacks. A different study also found invasive and noninvasive arterial blood pressures of 24 hour old Thoroughbred foals to be significantly higher than those of same age pony foals (Franco, Ousey, Cash, Rossdale, & Silver, 1986).

Parry et al. (1984), after surveying 296 clinically normal adult horses for resting coccygeal blood pressure, obtained uncorrected coccygeal values of 112,1 ± 16,5 mmHg for systolic pressure, 77,3 ± 14,3 mmHg for diastolic pressure and 88,9 ± 14,4 mmHg for MAP. According to Magdesian (2004) and Hardy (2010), normal NIBP values for adult horses are 111,8 ± 13,3 mmHg, ranging from 79 to 145 mmHg, for systolic pressures and 67,7 ± 13,8 mmHg, ranging from 49 to 106 mmHg, for diastolic pressures. A minimal MAP of 50 to 60 mmHg is essential for proper tissue, particularly cerebral, lung and coronary, perfusion (Magdesian, 2004).

Gay et al. (1977) evaluated the relation between systolic NIBP using the Doppler method and prognosis for 33 horses that underwent colic surgery. The authors showed how eight out of the nine horses that died had sistolic blood pressures between 59 and 99 mmHg, and the remaining

was a large mare in late pregnancy with a systolic blood pressure of 130 mmHg. From the surviving group only four horses had blood pressures inferior to 99 mmHg.

Parry, Anderson, & Gay (1983) also found a positive relation between low blood pressure and death in horses with colic, where 12 out of 13 (92%) of the horses with a systolic pressure inferior to 60 mm Hg died while only two out of 57 (4%) of the horses with systolic blood pressure above 100 mm Hg had the same outcome. In the same study, the mean systolic and diastolic pressures were 132,3 ± 3,8 mm Hg and 87,6 ± 2,9 mmHg, respectively, for the surviving group (n=66) versus 61,7 ± 8,4 mm Hg and 41,8 ± 6,9 mmHg for the non-surviving group (n=20).

Low arterial blood pressure in cases of abdominal colics can reflect a set of condictions such as hypovolemia, reduction of peripheral vasculature resistance, endotoxemia, SIRS and acid/base imbalances (Gay et al., 1977). High arterial blood pressures in abdominal pain are atributed mainly to pain and the presence of hypertension may be indicative that toxemia and hypovolemia haven't settled yet (Gay et al., 1977).

Probably due to peripheral vasoconstriction, noninvasive blood pressure measurements in hypovolemic patients may be falsely low, which makes direct blood-pressure measurements to probably be more accurate (Cook & Bain, 2003).

1.5. Oxygen delivery and uptake

Oxygen delivery (DO_2) is the quantity of oxygen delivered to the tissues from the heart per unit of time and it can be understood as the product of cardiac output (CO in mL/min) or cardiac index (CI in mL/Kg/min) and the arterial content in oxygen (CaO_2 in mL/dL).

The CaO_2 is normally distributed 98,5% in hemoglobin (Hb) and 1,5% dissolved or free (Divers, 2003a). Being that the dissolved portion of O_2 is so small we can calculate, with some accuracy, the CaO_2 as the product between the concentration of hemoglobin in the blood ([Hb] in g/dL), the quantity of oxygen transported by unit of fully saturated hemoglobin - which varies slightly according to the reference (1,34 mLO_2/g Hg (Magdesian, 2004) to 1,36 mLO_2/ g Hb (Allen & Holm, 2008)) - and the amount of oxygen-saturated hemoglobin known as oxygen saturation (SaO_2 %) (Allen & Holm, 2008):

DO_2 (mL/min) = CO x CaO_2 = (Stroke Volume x Heart Rate) x ([Hb] x 1,35 x SaO_2)

For a more accurate calculation of the CaO_2, Magdesian (2004), includes the arterial partial pressure of oxygen (PaO_2 mmHg), meaning the fraction of the total arterial gas pressure exerted by oxygen, and the free or dissolved portion of oxygen (0.0031) in the equation:

CaO_2 (mL/dL) = [Hb] x 1,34 x SaO_2 + (PaO_2) 0,0031

Two studies established normal values of DO_2 for healthy adult ponies and foals as 9,45 ± 1,92 mL/Kg/min and 31 mL/Kg/min respectively (Magdesian, 2004).

Oxygen uptake or consumption (VO_2) and oxygen extraction ratio (OER) represent the amount of oxygen that is being extracted from the microcirculation blood and used by tissues as an exact value or a percentage of DO_2, respectively (Magdesian, 2004). The OER is about 23 to 30% of the DO_2 (Divers, 2003a) and, under normal conditions, it stays constant under a large interval of DO_2 values since these are always higher than the VO_2 (Allen & Holm, 2008; Magdesian, 2004).

The VO_2 can be calculated as the difference between the arterial and venous oxygen content (CvO_2) multiplied by the CO (Magdesian, 2004):

$$VO_2 \text{ (mL/min)} = (CaO_2 - CvO_2) \times CO$$

The CvO_2 is calculated the same way as the CaO_2 but using the venous oxygen saturation and concentration values (Magdesian, 2004).

Two studies established normal values of VO_2 for healthy adult ponies and foals as 2,05 ± 0,74 mL/Kg/min and 5,6 mL/Kg/min respectively (Magdesian, 2004).

To calculate the oxygen extraction ratio the CO does not need to be known (Magdesian, 2004):

$$OER\ (\%) = ([CaO_2 - CvO_2]\ 100) / CaO_2$$

A study done in five healthy foals determined the OER as 18,0 ± 0,02% (Magdesian, 2004).

VO_2 can become dependent of the DO_2 if the last decreases below a certain point like in anemia, hypovolemia, low CO, etc. (Magdesian, 2004). In these cases, tissues respond by increasing the OER (Allen & Holm, 2008). When the DO_2 reaches a point where the OER can no longer be increased to secure appropriate tissue oxygenation and aerobic metabolism, called critical DO_2 (Allen & Holm, 2008), the affected tissues enter a state of dysoxia (Magdesian, 2004) and lactate production is initiated.

1.6. Lactate

Lactate measurements have been used since the mid-1970's in equine critical care but real advances concerning the use of lactate in this area have only been made in the last 20 years (Franklin & Peloso, 2006). Lactate is the ionized form of lactic acid and a fairly quick and easy biomarker to measure in either whole blood or plasma. It can provide important information about peripheral perfusion and O_2 delivery to the tissues (Magdesian, 2004), as well as help decide about diagnosis and treatment options. In addition, lactate is also an important indicator in equine medicine when it comes to therapy monitoring and prognosis evaluation, particularly in neonates and colic patients (Allen & Holm, 2008; Radcliffe, Buchanan, Cook, & Divers, 2015; Tennent-Brown, Wilkins, Lindborg, Russell, & Boston, 2010).

Glycolysis is an anaerobic process that all cells are able of performing, being that brain, liver and skeletal cells are the ones with higher rates. Glycolysis is the first step of glucose metabolism and it happens in the cell's cytoplasm (Allen & Holm, 2008). The result of each molecule of glucose metabolized are two molecules of pyruvate, two molecules of nicotinamide adenine dinucleotide (NADH) (by reduction of the enzyme cofactor NAD^+), two molecules of adenosine triphosphate (ATP), two molecules of water and two hydrogen ions (Nelson & Cox, 2013):

$$C_6H_{12}O_6 + 2NAD^+ + 2ADP + 2P_i \rightarrow 2CH_3COCOO^- + 2NADH + 2ATP + 2H_2O + 2H^+$$

The pyruvate molecules resulting from glycolysis, still contain most of the biologically available energy from the glucose molecule. Under normal O_2 conditions, in the mitochondria of eukaryotic cells, this energy can be extracted by oxidizing the pyruvate to acetate which then enters the citric acid cycle and is oxidized to CO_2 and H_2O (Nelson & Cox, 2013). Still in the mitochondria, NADH is reoxidized to NAD^+ by transferring their electrons to the respiratory chain which provides energy for the production of ATP by mitochondrial oxidative phosphorylation (Nelson & Cox, 2013):

The entire oxidation of a molecule of glucose results in the production of 38 molecules of ATP (Allen & Holm, 2008; Nelson & Cox, 2013).

When a cell does not possess mitochondria, such as erythrocytes, retina or brain cells (Nelson & Cox, 2013), the pyruvate is converted into lactate by the enzyme lactate dehydrogenase (LDH) and, at the same time, the NADH is oxidized back to NAD^+ allowing for glycolysis to continue (Allen & Holm, 2008):

$$CH_3COCOO^- + NADH + H^+ \leftrightarrow CH_3CHOHCOO^- + NAD^+$$

Cells from liver, kidney and heart are capable of using the lactate produced by these cells and other tissues such as extremely active skeletal muscle as a source of energy. This process is called the Cori cycle (Allen & Holm, 2008; Nelson & Cox, 2013). The lactate is transported to these tissues and converted back to pyruvate which then enters the cell's mitochondria to produce ATP. In the liver and kidney, lactate can also be transformed into glucose through gluconeogenesis and either stored under the form of glycogen or released into the blood to be used by other tissues (Allen & Holm, 2008). Under normal conditions, the metabolism of lactate consumes hydrogen ions and generates carbon dioxide that is in equilibrium with bicarbonate. Therefore lactate metabolism has a final alkalinizing effect (Magdesian, 2004).

Glycolysis is a much faster but less efficient method of producing energy than the aerobic metabolism (Allen & Holm, 2008). Anaerobic metabolism requires about 18 times more glucose to be metabolized to produce the same amount of ATP as it would under aerobic conditions (Nelson & Cox, 2013).

In the absence of appropriate O_2 levels in the tissues, termed hypoxia, the cell must find an alternative way of reducing NADH to NAD^+ (Nelson & Cox, 2013). This is achieved by the reduction of pyruvate to lactate by LDH as described before for cells that don't have mitochondria.

The reduction of NAD^+ to NADH and the consumption of ATP result in the production of hydrogen ions which start to accumulate instead of being consumed by oxidative phosphorylation, as would happen in aerobic conditions (Allen & Holm, 2008).

The capacity of converting lactate to pyruvate or glucose will also be affected by the level of hypoxia present in such tissues (Allen & Holm, 2008). As hypoxia persists, lactate will start to accumulate inside the cell until it reaches concentrations high enough for it to start crossing the cell membrane into the blood stream (Allen & Holm, 2008). When the production of lactate by the cells exceeds the clearance rates, mainly by the liver and kidneys, hyperlactemia develops (Tennent-Brown et al., 2010; Radcliffe et al., 2015). At first the hydrogen ions are titrated by body buffers but, as these get depleted, acidemia starts developing alongside with lactate accumulation (Allen & Holm, 2008).

The lactate produced by anaerobic cellular metabolism is the levorotatory isomer, better known as *L*-lactate.

D-lactate is the lactate isomer produced by the glucose metabolism of bacteria since mammalian cells do not have the D-lactate dehydrogenase enzyme (Nappert & Johnson, 2001).

Although hyperlactemia due to increases in *D*-lactate is rare in monogastric mammals, *D*-lactic acidosis has been reported in humans with short bowel syndrome, in a cat with exocrine pancreatic insufficiency and intestinal bacteria overgrowth (Allen & Holm, 2008) and cats with Diabetes Mellitus (Nappert & Johnson, 2001). *D*-lactate increases are more significant in ruminants. *D*-lactic acidosis has been shown to be common in calves with acidosis due to diarrhea and in adult ruminants subjected to grain overload where the production of *D*-lactate by *Lactobacillus* spp overcomes the production of *L*-lactate (Nappert & Johnson, 2001).

D-lactate is excreted in the urine although renal clearance is much higher for the *L* isomer (Nappert & Johnson, 2001). Some *D*-lactate can be transformed in pyruvate and then oxidized or used for gluconeogenesis (Nappert & Johnson, 2001).

Commercial lactate analyzers are only able of detecting the *L*-lactate isomer, therefore, increases in *D*-lactate should be suspected in patients with gastrointestinal disease and acidosis but with normal *L*-lactate levels (Allen & Holm, 2008). This is due to the production of *D*-lactate by intestinal bacteria, mainly from the large intestine, which is absorbed and detected in the blood pH (Franklin & Peloso, 2006).

Lactate concentrations can be measured using whole blood or plasma in commercial analyzers, preferably those that have been validated for the species in focus (Magdesian, 2004; Corley,

2008b). One should be aware that lactate concentrations tend to be lower when using a whole blood sample, most likely due to the dilutional effect of the erythrocytes, which are poorer in lactate than plasma (Radostits, Gay, Hinchcliff, & Constable, 2007). Other lactate analyzers work by measuring colorimetric changes which can be affected by high hematocrits (Corley, 2008b).

In situations where a commercial lactate analyzer is not available, given that the concentration of plasma proteins is normal, plasma lactate concentration can be known with relative accuracy by calculating the anion gap (AG) in adult horses (Smith, 2009):

$$AG = (Na^+ + K^+) - (Cl^- + HCO_3^-)$$

Still, the estimation of lactate concentration through the AG should be used with caution since the AG can also be elevated in cases of azotemia and decreased in hypoalbuminemia situations (Tennent-Brown, 2015).

Increases in *L*-lactate may be due to several causes. In veterinary medicine hyperlactemia can be divided into two categories: type A and type B (Magdesian, 2004; Allen & Holm, 2008; Radcliffe et al., 2015).

Radcliff et al, 2015, defined type A hyperlactemia as the one resulting from tissue hypoxia secondary to deficient Do_2 and type B hyperlactemia when it's caused by mitochondrial dysfunction, altered carbohydrate metabolism or decreased lactate clearance.

A more thorough description of the possible causes of hyperlactemia separated into different categories is presented in Table 3.

Table 3: Types of lactic acidosis in veterinary medicine. Adapted from Magdesian (2004), Allen and Holm (2008) and Radcliffe (2015).

Type A: Deficient DO_2	Type B		
Exercise	B1: Underlying disease	B2:Drugs/toxins	B3:Mitochondrial disease
Seizures	Liver disease	Ethylene glycol	Mitochondrial myopathies
Hypoxia	Diabetes mellitus	Propylene glycol	Inborn
Hypotension	Sepsis	Epinephrine	Acquired
Anemia	Renal failure	Carbon monoxide	
Shock: cardiogenic, hypovolemic, septic	Neoplasia	Salicylates	
Regional hypoperfusion	Alkalosis	Acetaminophen	
Global hypoperfusion	Acutely injured lung		
Carbon monoxide exposure			

The clinical presentation of hyperlactemia is the one of the resulting metabolic acidosis but usually the clinical signs of the underlying condition causing the increases in lactate are the most evident (Smith, 2009).

In horses, the most common causes for hyperlactemia are consequence of cellular hypoxia (type A) in cases of hypovolemia, hypotension, low arterial O_2 content and hypermetabolic states such as increased muscle activity, sepsis or seizures (Magdesian, 2004).

Activated white blood cells and catecholamine increases during SIRS, sepsis-induced inflammatory mediators, inhibition of pyruvate dehydrogenase, glucose infusions and systemic alkalosis all contribute for the production of lactate under aerobic conditions for multiple reasons (Magdesian, 2004).

One study showed that postpartum and lactating mares may have slightly elevated lactate concentrations with values ranging from 0,59 ± 0,17 mmol/L to 1,82 ± 0,77 mmol/L. Foals under 24h of age tend to have higher plasma lactate concentrations which quickly normalize after 24h of age (Magdesian, 2004). Two studies demonstrated that postpartum foals had a concentration of 3,0 ± 0,4 mmol/L (Silver et al. 1987) and found lactate levels for afterbirth, 12h and 24h old foals to be 4,9 ± 1,02 mmol/L, 2,25 ± 0,6 mmol/L and 0,89 mmol/L respectively (Kitchen & Rossdale, 1975). Attention should be payed when handling and restraining foals and adult horses since exercise, struggling and stress can cause temporary elevations in blood lactate levels (Magdesian, 2004).

Some dogs and humans with lymphoma have been reported to be hyperlactemic or have hyperlactemia following LRS administration. Although such has not yet been reported for horses, neoplasia should be considered for horses with unexplained hyperlactemia (Allen & Holm, 2008; Magdesian, 2004).

Normal values for adult horses are generally considered to be less than 1,5 mmol/L (Fielding & Magdesian, 2005; Nappert & Johnson, 2001) or 2,0 mmol/L (Magdesian, 2004). Two studies, mentioned by Magdesian (2004), performed in normal horses established normal blood lactate levels as 1,0 ± 0,3 mmol/L (0,7 – 1,7 mmol/L) and 1,04 ± 0.26 mmol/L (0,57 – 1,53 mmol/L). Plasma lactate concentrations can be considered as mild: 2,5 - 4,9 mmol/L; moderate: 5 - 9,9 mmol/L; and severe: >10,0 mmol/L (Nappert & Johnson, 2001). While hyperlactemia is considered a mild increase in blood lactate concentration to 2 – 5 mmol/L, lactic acidosis is considered present when lactate concentrations in the blood rise above 5 mmol/L resulting in metabolic acidosis (Franklin & Peloso, 2006). Increases in plasma lactate have proved to have a strong correlation with the patient's prognosis according to different authors in both human and veterinary medicine.

In human medicine, intensive care physicians have concluded that changes in lactate concentration and lactate clearance during the first 24h provided the most information, prognosis

wise (Franklin & Peloso, 2006). Two studies demonstrated that early lactate normalization in the first 6 hours after resuscitation showed to have the strongest relation to survival of patients with sepsis or septic shock and that serial plasma lactate concentrations were more important than the lactate concentration at admission in predicting multiple organ failure and mortality in septic shock (Radcliffe et al., 2015).

Several studies have been conducted to assess the relevance of lactate in sick dogs. One of these measured the lactate concentrations in 109 sick dogs and 20 healthy dogs concluding that 95% of the animals in the sick group had plasma lactate concentrations higher than the normal reference values and higher than the median concentrations of the healthy dogs group. 25% of the sick dogs died or were euthanized and the median lactate concentration of these animals was higher than both the healthy and the sick survivor groups (Allen & Holm, 2008).

A retrospective study on the relationship between gastric necrosis and plasma lactate concentration, obtained before treatment, in dogs with gastric dilation-volvulus concluded that gastric necrosis was present in 74% (23/31) of the dogs with lactate concentrations >6.0 mmol/L and only in 21% (15/71) of the dogs with < 6 mmol/L. Only 58% (18/31) of the dogs in the first group survived while 99% (69/71) of the dogs in the second group survived. Still in this study, gastric necrosis was present in 80% (16/20) of the dogs with lactate concentrations > 7 mmol/L, 92% (11/12) of dogs with lactate concentrations > 8 mmol/L and 100% (7/7) of dogs with lactate concentrations > 10 mmol/L (Allen & Holm, 2008).

Two studies done in dogs with babesiosis found plasma lactate concentrations to be a strong indicator of severity of illness, poor clinical presentation and prognosis, level of parasitemia and alterations in other blood biomarkers (Allen & Holm, 2008).

In equine medicine, lactate measurements have proven to be of similar importance as in human medicine in assessing the patient's condition and prognosis. Multiple studies have been done to clear the role of lactate measurements in equine critical care medicine. Equine colic is one of the main situations where lactate measurements may come in use. Less than 25% of the horses with colic that had plasma lactate concentrations greater than 11,2 mmol/L survived in comparison to 93% of horses with colic and lactate measurements ranging from 0,0 to 8,3 mmol/L (Magdesian, 2004).

The importance of serial lactate measurements as an estimation of prognosis is clear. A study by Johnston et al. (2007), on horses with a ≥ 360° large colon volvulus demonstrated that surviving horses had significantly lower plasma lactate concentrations at admission time than the non-surviving ones: 2,98 ± 2,53 mmol/L versus 9,48 ± 5,22 mmol/L respectively, and that the same happened for horses with a viable colon compared to nonviable colon: 3,3 ± 2,85 mmol/L versus 9,1 ± 6,09 mmol/L respectively. In the same study the authors also reported that the lactate levels 24h after surgery had returned to normal values of 0,96 ± 0,60 mmol/L in the

horses that survived but remained significantly elevated for those who did not survive, with values of 3,24 ± 3,08 mmol/L.

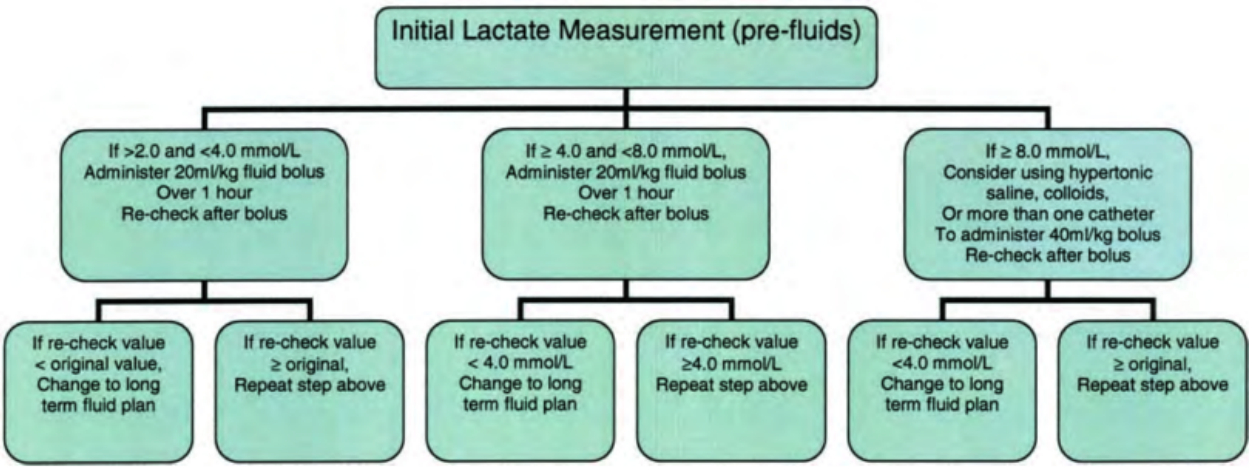
Another study on horses undergoing abdominal surgery showed that those with not only marked increases in plasma lactate before (> 7,5 mmol/L) but also with abnormal or increasing concentrations after surgery, had a guarded prognosis for survival (Radcliffe et al., 2015). In the same way, Tennent-Brown et al. (2010), also showed that horses that died or were euthanized had a significantly higher lactate at admission than the surviving ones: 4 mmol/L (range 0,6-18,2 mmol/L) versus 1,3 mmol/L (range 0,3 – 13,9 mmol/L), respectively. In the same study, large intestinal strangulating disease and colitis seemed to be have the strongest relation between elevated lactate and poor outcome.

Although an evident relation between blood lactate concentrations and prognosis exists, some authors defend that special care should be taken when providing a diagnosis based solely on lactate values before treatment. The authors give the example of hypovolemic horses with colitis or hemorrhagic shock that present with considerable increases in lactate and respond well to aggressive fluid therapy, showing no relation between outcome and initial lactate values (Fielding & Magdesian, 2005).

Curiously, in opposition to horses, ponies and miniature horses tend to have higher blood lactate concentrations at admission compared with horses but these haven't proven useful in assessing the need for surgery, distinguishing strangulating from non-strangulating lesions and concluding about the risk of non-survival (Radcliffe et al., 2015).

Although blood lactate concentrations may provide some information about the cause of colic, it is mainly an indicator of the general state of oxygen supply to tissues and oxygen consumption by anaerobic metabolism. Therefore, plasma lactate is mainly useful to estimate the severity and extent of shock as well as the response to therapy (Radcliffe et al., 2015).

Plasma lactate concentrations at admission time and during the following hours/days have also shown to be strongly associated with prognosis in sick foals. Newborn foals with higher lactate concentrations usually have a story of dystocia or premature placental separation and/or are premature/dysmature foals. The most common diagnosis for foals with increased blood lactate are of sepsis, enterocolitis, enteritis, colic, trauma, respiratory disease, immune-related disease and encephalopathy (Radcliffe et al., 2015).Hyperlactemia and lactic acidosis should be treated



with large volumes of crystalloid replacement fluids. Fielding and Magdesian (2005) created an algorithm on how to use blood lactate concentrations as a guide for fluid therapy in adult horses (Figure 16).

In cases of hyperlactemia due to hypoperfusion states, it is expected that, with adequate fluid therapy and volume resuscitation, the blood lactate concentrations will decrease fairly quickly.

This algorithm was created mainly as an aid for field therapy and the authors insist that a detailed physical exam should be performed before initiating fluid therapy and that horses whose lactate concentrations keep increasing or did not reach values < 4 mmol/L after the third 20L fluid bolus may need additional laboratory evaluation and should be referred to a hospital.

When hyperlactemia persists after fluids are administered, other causes such as severe inflammation, blood loss or sepsis should be considered (Radcliffe et al., 2015). Close attention should be payed to the presence of conditions responsible for increases in blood lactate but that do not beneficiate from the administration of large amounts of fluids such as heart failure or urinary obstructions (Fielding & Magdesian, 2005).

Some may feel tempted to administer bicarbonate to lactic acidotic patients. Even though this will, in fact, correct the blood pH, it will not resolve the underlying cause of hyperlactemia and it might worsen the situation by causing a hypernatremic alkalosis on an already metabolically unbalanced patient (Smith, 2009).

1.7. Creatinine and Urea

Creatinine is a molecule resultant from the degradation of creatine and creatine-phosphate during muscle metabolism and excreted by the kidneys (Braun & Lefebvre, 2008; Radcliffe et al., 2015). The skeletal muscle contains about 95% of the creatine and creatine-phosphate in the body although animals and humans can also obtain these molecules from meat in their diet (Braun & Lefebvre, 2008). Creatinine exists mainly as a free form in the plasma and is freely filtrated by the glomerulus but not reabsorbed in any part of the nephron, explaining why it represents a standard marker for estimating glomerular filtration rate and assessing kidney function (Braun & Lefebvre, 2008; Radcliffe et al., 2015). Although no kidney secretion of creatinine has been reported in cats or ponies, it is believed that creatinine is secreted by the renal tubules in horses and to a smaller scale in humans, rabbits, sheep and pigs (Braun & Lefebvre, 2008; Hollis & Corley, 2008).

Even though plasma creatinine has been largely used to evaluate kidney function it is a weak marker for early loss of kidney function since it only rises after about 75% of the nephrons have lost their function (Radcliffe et al., 2015; Schott II, 2010). Still, a doubling of creatinine concentration is considered to correspond roughly to a 50% decrease of glomerular filtration rate (Schott II, 2010). Despite indicating a reduced glomerular filtration rate, serum creatinine levels alone are of little help when trying to understand the underlying cause for renal dysfunction

(Radcliffe et al., 2015). Never the less, plasma creatinine is a helpful tool to guide and monitor therapy and to estimate the prognosis, especially for critical care patients (Radcliffe et al., 2015).
Normal serum creatinine concentrations for adult horses range from 0,9 to 1,9 mg/dL (Fielding, 2015) or 2 mg/dL (Orsini & Divers, 2014) although it has been reported that normal adult Quarter horses and Warmbloods may have creatinine levels up to 2,4 mg/dL (Orsini & Divers, 2014; Radcliffe et al., 2015).
Some newborn foals may have creatinine concentrations of 5 to 8 mg/dL and up to 13,6 mg/dL while maintaining a normal kidney function (Orsini & Divers, 2014; Radcliffe et al., 2015). These values have been related to foals born to mares with placental dysfunction, especially if blood urea nitrogen levels are normal , and with being bathed in amniotic fluid with abnormally high creatinine concentrations (Corley, 2008a; Schott II, 2010; Schaer & Orsini, 2014; Radcliffe et al., 2015), and are expected to decrease to normal values within two to five days after birth in the absence of further complications (Corley, 2008a; Schott II, 2010; Schaer & Orsini, 2014). After the first days of life, the foal's serum creatinine concentrations might be lower than the ones of an adult horse, probably due to their rapid growth and smaller proportion of skeletal muscle to body weight (Schott II, 2010).
Renal hypoperfusion and nephrotoxic substances are the most common causes for acute kidney failure in horses and creatinine levels should be monitored whenever the patient's in risk of one of these, as well as in situation where urinary tract obstruction or rupture is present or suspected (Radcliffe et al., 2015).
Creatinine concentrations up to 3,5 mg/dL are common in moderately to severely dehydrated or hypovolemic horses (Cook & Bain, 2003; Corley, 2008a) but these values can reach 8 mg/dL in these animals (Radcliffe et al., 2015). However, high normal values ranging from 1,5 to 1,8 mg/dL might be an indication of subclinical hypovolemia and should be taken into account along with clinical history, physical exam and other laboratory results (Corley, 2008a).
Diarrhea and other gastrointestinal diseases, endotoxemia and septic shock, acute blood loss, hypovolemic shock, thrombotic episodes, coagulopathy, acute heart failure, and prolonged exercise are some of the most common causes of vasomotor nephropathy (Schott II, 2010; Radcliffe et al., 2015). Increased creatinine values due to kidney hypoperfusion are expected to correct within a 36 hour period following correction of fluid deficits (Schaer & Orsini, 2014; Radcliffe et al., 2015). One should suspect actual kidney damage if creatinine levels do not decrease as expected with appropriate fluid administration (Corley, 2008a).
Several authors agree that serial measurements of serum creatinine for patients in risk of developing acute kidney injury is of the utmost importance and that an increase of 0,3 mg/dL or greater in creatinine serum concentration, even if it remains within normal limits, is considered a

significant indicator for the developing of nephropathy (Divers, 2003b; Fielding, 2015; Radcliffe et al., 2015).

Urea is the main form of nitrogen elimination in mammals. It is a small molecule synthetized in the liver from bicarbonate and ammonium and released into the sinusoidal blood as blood urea nitrogen (BUN) (Barton, 2010). Blood urea is freely filtered by the glomerulus and reabsorbed in the collecting tubule (Braun & Lefebvre, 2008). Urea reabsorption increases when urine flow within the tubule is reduced which may explain higher urea levels in dehydrated or hypovolemic animals (Braun & Lefebvre, 2008).

Normal levels of blood urea nitrogen in adult horses range from 10 to 24 mg/dL according to Orsini and Divers (2014) or from 17 to 27 mg/dL according to Hollis (2008).

Diet protein can represent a significant source of ammonium for the production of urea and low concentrations of blood urea nitrogen might be explained by low-protein diets or fasting (Braun & Lefebvre, 2008; Hollis & Corley, 2008). However, after a prolonged fasting, BUN levels rise, possibly due to body protein catabolism (Braun & Lefebvre, 2008). Schott II (2010) wrote that high-protein diets may cause a twofold or greater increase in BUN concentration and that there was a 50% or higher increase in blood urea after prolonged exercise in horses, possibly explained by a reduction in renal blood flow accompanied by an increase in protein catabolism. Conversely, Ainsworth (2002) affirms that increases in BUN in horses are rarely related to body protein catabolism or high-protein diets.

Other explanation for low blood urea nitrogen concentrations is hepatic failure where the liver is incapable of converting ammonia into urea (Durhan, 2008; Barton, 2010). Decreases in BUN due to liver failure are usually accompanied by increases in blood ammonia (Schott II, 2010). Although BUN concentrations have been found to be within normal limits in the majority of horses with hepatopathy, low BUN values in these animals usually relate to a poor prognosis (Durhan, 2008). Low BUN is also commonly found in young foals after situation of anabolic demand for aminoacids (Schott II, 2010) and in animals receiving anabolic steroids (Corley & Stephen, 2008).

There are numerous reasons for increases in BUN and, besides the already mentioned increases from diet and protein catabolism. Causes related to kidney function are the most common ones (Hollis & Corley, 2008). Therefore, analyzing creatinine and BUN values simultaneously is of greater value when assessing kidney function.

The term azotemia is used to describe the increase in blood creatinine and urea and it can be considered to be pre-renal, renal or post-renal whether the causes for such increments are due to decreased kidney perfusion, kidney disease or urinary tract damage or obstruction, respectively (Hollis & Corley, 2008; Schott II, 2010). A BUN/creatinine ratio greater than 15:1 according to Divers (2003b) or greater than 20:1 according to Radcliffe et al. (2015) should be

indicative of pre-renal azotemia because of an increased urea reabsorption in cases of reduced urine flow in the proximal tubules. An increased BUN/creatinine ratio may also be indicative of post-renal azotemia due to urea absorption through the peritoneum in case of urinary tract rupture or due to reduced urine flow in obstructions (Radcliffe et al., 2015; Schott II, 2010). In some cases of acute renal failure secondary to diarrhea, blood urea nitrogen values may be normal or slightly elevated, due to protein loss, while creatinine values are greatly increased (Schaer & Orsini, 2014).

In contrast, values inferior to 10:1 for the same ratio are more likely present in renal azotemia since the excretion of both molecules and the reabsorption of urea is impaired (Radcliffe et al., 2015; Schott II, 2010).

Some drugs administered to horses can result in serious nephrotoxic injury, especially in critically ill and dehydrated/hypovolemic patients whose kidney function might already be deranged. Aminoglycoside or tetracycline antimicrobials, NSAIDs and norepinephrine are some of the drugs commonly used in equine medicine that carry nephrotoxic properties (Hollis & Corley, 2008; Schaer & Orsini, 2014; Radcliffe et al., 2015; Fielding, 2015b). Particular attention should be payed to NSAIDs that are often administered to horses suffering from gastrointestinal disease, sometimes as repeated doses, many times before proper evaluation of fluid balance (Hassel, 2015). Polymyxin B can also exert nephrotoxic effects in its antimicrobial concentrations although when used in endotoxin-biding doses this risk is significantly reduced, yet not discarding the need for monitoring kidney function (Ainsworth, 2002; Hollis & Corley, 2008; Schott II, 2010).

Pigments like hemoglobin and particularly myoglobin may also be responsible for the onset of acute renal failure, particularly after tying-up or postanaesthetic myopathy episodes (Hollis & Corley, 2008; Schaer & Orsini, 2014). Other causes of acute renal failure include leptospirosis infections and intoxication with cantharadin from blister beetles, heavy metals, red maple or vitamin D (Hollis & Corley, 2008; Fielding, 2015b).

In horses suffering from abdominal colic, higher blood concentrations of creatinine and urea proved to be related with increased mortality (Buchanan, 2014). A study involving 167 horses suffering from colic or colitis found that horses with azotemia that lasted longer than 72 hours had three times more chances of dying or being euthanized (Groover, Woolums, Cole, & LeRoy, 2006).

1.8. Total plasma proteins, total solids and albumin

The plasma's protein content has proved to be an important tool in assessing volemia status as long as pathologic protein losses or gains are excluded (Ethell, Dart, Rose, & Hodgson, 2000; Magdesian, 2015a). While total plasma proteins (TP) are measured by a chemistry analyzer, plasma's total solids (TS) only require a refractometer making it easier, cheaper and faster to

measure, especially in field conditions (Magdesian, 2015). On the other hand, total solids measurements only represent the total protein concentration considering the remaining plasma components are within normal concentrations, for example, hyperlipidemia can cause an increase in total solids up to 2.0 g/dL (Weiser, 2012). The influence of serum glucose and urea concentrations in the measurement of total solids is negligible (Weiser, 2012).

The three major constituents of plasma proteins are albumin, globulins and fibrinogen (Ethell et al., 2000).

Albumin is synthesized in the liver and is the main protein responsible for maintaining the plasma's oncotic pressure (Frandson, Wike, & Fails, 2009; Fielding, 2015). Many ions, hormones and drugs circulate binded to albumin, preventing their fast excretion through the glomerulus (Corley, 2008a; Frandson, Wike, et al., 2009). Albumin also works as a carrier of water-insoluble compounds in the plasma and, along with globulins and phosphate, is one of the main contributors for the buffer base in plasma (Corley, 2008aCorley, 2008aPalmer, 2015)Palmer, 2015).

Normal albumin concentrations for horses are 2,3 – 3,6 g/dL according to Corley & Stephen (2008) or 2,9 - 3,8 g/dL according to Orsini & Divers (2014). Hyperalbuminemia in horses only seems to occur in severe dehydration (Corley & Stephen, 2008). Hypoalbuminemia may be due to chronic inflammatory disease, amyloidosis, tuberculosis, colitis, parasitism, starvation or other causes of protein loss mentioned below (Corley & Stephen, 2008; Mair, 2002a). Albumin concentrations inferior to 1 - 1,5 g/dL have been anecdotally considered the limit for edema formation in horses but there is still no scientific data to support these values (Fielding, 2015).

Although hypovolemia causes the concentration of these proteins to increase more or less proportionally, there are other causes that may be responsible for the increase of just globulins or fibrinogen. Hyperglobulinemia may be present in response to chronic antigen stimulation or inflammation, for example in parasitism or closed-cavities infections like abdominal or thoracic abscesses (Corley & Stephen, 2008; Ethell et al., 2000), and hyperfibrinogemia is indicative of an active inflammatory or infectious disease (Corley & Stephen, 2008).

Many common diseases in equine medicine result in protein depletion and, as mentioned before, the use of total plasma protein concentration is only reliable provided that there are no significant ongoing or recent protein losses (Ethell et al., 2000; Magdesian, 2015a). Causes for decreases in total plasma protein's concentration, besides the mentioned above for hypoalbuminemia, are GI ulceration, strangulating GI obstruction and protein-losing enteropathies, protein-losing nephropathy or glomerulonephritis, liver disease, acute blood loss and acute peritonitis or pleuritis (Ethell et al., 2000; Corley & Stephen, 2008; Hardy, 2010). Other causes for low plasma protein concentration that are not mandatorily related to morbid states include fluid overload, excess of water intake and severe malnutrition (Corley & Stephen,

2008; Hardy, 2010). Animals suffering from protein losses may, therefore, be dehydrated and still present normal plasma protein concentrations whereas the opposite may be observed in animals with increases in globulins and fibrinogen who are normovolemic (Ethell et al., 2000).

Because protein levels in plasma are not altered by stress or excitement, as long as the conditions mentioned above are excluded, total plasma protein or total solids are often a better guide for extracellular fluid volume conditions than the hematocrit (Ethell et al., 2000).

Normal plasma protein values for adult horses range from 5,2 to 7,2 g/dL according to Corley and Stephen (2008) or 5,5 to 7,5 g/dL according to Van Harraveld and Gaughan (2002). For foals normal plasma protein values go from 4,2 to 7,8 g/dL (Corley & Stephen, 2008).

Orsini and Divers (2014) consider hypovolemia according to TP as mild for 7,0 - 8,0 g/dL, moderate for 8,0 - 9,0 g/dL and severe for TP values above 9 g/dL. Rose and Hodgson (2000) consider that horses with TP values under 7,5 g/dL don't require fluid therapy while animals with TP values ranging from 7,5 to 8.5 g/dL have indication for IV fluids and horses with plasma protein concentrations greater than 8,5 g/dL definitely require intravenous fluids.

Two studies experimenting the alterations of PCV and TP in horses deprived of water for three and eight days found that there were very little changes in PCV at the end of that period whereas TP levels increased significantly in both studies (Magdesian, 2015).

Sequential measurements of plasma proteins provide the most reliable information about the patient's evolution and therapy success (Corley & Stephen, 2008).

Plasma protein concentrations of less than 4 g/dL have been considered the limit for edema formation in horses (Fielding, 2015).

In horses with colic, plasma protein may be low due to sequestration into the abdominal cavity or intestinal lumen secondary to peritonitis or enteritis, respectively (Van Harraveld & Gaughan, 2002). A study conducted by Parry et al. (1983), on individual variables used in case assessment as a way to define prognosis in colic cases, did not find significant differences between the total plasma proteins of the surviving and nonsurviving groups, however, the authors explain that all horses with less than 5,2 g/dL total proteins at admission died.

Mair and Smith (2005) studied the association between several baseline variables at admission and the development of post-operative shock in 252 horses undergoing colic surgery. The authors found that 36,8% of the horses with TP greater than 9 g/dL developed post-operative shock, while the same only happened for 13,3%, 15,6%, 7,0% and 16,7% of the horses with TP of 8–8,9 g/dL, 7–7,9 g/dL, 6–6,9 g/dL and < 6 g/dL, respectively.

1.9. Packet cell volume

Packed cell volume (PCV) or hematocrit represents the portion of blood composed by erythrocytes (Weiser, 2012). PCV can easily be measured using the microhematocrit technic, considered to be the most accurate technic by Tennent-Brown (2015), or using an automatic cell

count machine (Sellon, 2010; Weiser, 2012). As what happens for total plasma protein or total solids, measurement of PCV is one of the most frequently conducted tests in equine practice and can be useful in assessing hydration status at admission and help monitor therapy through sequential measurements (Corley, 2008a; Ethell et al., 2000). The biggest downfall of PCV is that it can be affected by splenic contractions in response to stress or excitement or in the presence of real polycythemia ((Rose & Hodgson, 2000; Sellon, 2004Corley, 2008; Nolen-Waltson et al., 2011;).

There also seems to exist a considerable variation in PCV among different types (Table 4) and breeds of horses as well as among animals of the same breed (Sellon, 2010; Tennent-Brown, 2015). For example, a review of anemia in horses showed Thoroughbred horses to have a normal PCV of 41% ± 3,8 while Clydesdales had a PCV of 33% ± 3 (Sellon, 2010).

Table 4: PCV values according to type of horse. Adapted from Sellon (2010).

	Light horse	Draft horse	Miniature horse
PCV (%)	32-50	24-44	24-42

There are several reasons for PCV to decrease, resulting in anemia. These may be classified into regenerative anemias such as the ones caused by hemorrhage and hemolysis or classified as nonregenerative anemias like the ones secondary to iron deficiency, chronic inflammation, neoplasia, endocrine diseases and generalized bone marrow failure (Sellon, 2010), besides iatrogenic fluid overload (Fielding, 2015). One must be aware that the presence of anemia simultaneously to hypovolemia may originate a falsely low or normal PCV (Sellon, 2010).

Pathologic rises in PCV happen in cases of acute blood loss and hypovolemia (Sellon, 2010). A PCV greater than 45% usually reflects contraction of the plasma volume (Ethell et al., 2000) and non-exercising horses with hematocrits above 50% are almost always hypovolemic (Corley, 2008a), although Orsini and Divers (2014) consider the normal PCV values for adult horses to range from 32% to 53%. Some authors doubt the sensitivity of PCV as a hydration status indicator. Two studies that deprived horses from water for three and eight days showed no significant changes in PCV at the end of that period whereas TP levels increased significantly in both studies (Magdesian, 2015).

Van Harraveld and Gaughan (2002) link a PCV greater than 65% to a poor prognosis in horses with colic. Proudman, Smith, Edwards, & French (2002b) compared the PCV between horses surviving colic surgery and those dying or being euthanized prior to recovering from anesthesia, and concluded that baseline PCV for the surviving group was of 38 ± 8% whereas for the nonsurviving group was of 50 ± 11%. A study conducted by Ihler, Venger, and Skjerve (2004) found that, for horse's that were treated medically for colic, the surviving group had a mean PCV

of 34%, ranging from 22 to 50%, while the nonsurviving group presented a mean PCV of 48%, ranging from 33 to 69%.

Mair and Smith (2005) studied the association between several baseline variables at admission and the development of post-operative shock in 252 horses undergoing colic surgery. The authors found that 52,4% of the horses with a PCV greater than 50% developed post-operative shock, while the same only happened for 14,3%, 8,9% and 5,4% of the horses with PCV of 40-49%, 30-39% and < 30%, respectively.

A study by Proudman, Edwards, Barnes, and French (2005) analyzing 275 horses undergoing surgery for large intestinal disease found PCV values on admission to be significantly and positively related to post-surgery mortality. Later, Proudman et al. (2006) analyzed 774 equine surgical colic cases and confirmed that there was a strong relation between PCV at admission and intra or post-surgery mortality but this was not a linear relation. The authors explain the existence of an “optimum” PCV interval and the further the horse’s values are from this interval, the worse the prognosis for survival. Another study looked at the association of capillary refill time, PCV and pain with mortality in 152 horses and found that the mortality rate was higher (69%) in horses with a PCV greater than 54%, although these were not statistically significant results (Dukti & White, 2009).

CHAPTER II

2.1. Goals

The present work is a preliminary study that aims to determine the existence of a beneficial effect in administrating a 2 litter bolus of 7,2% hypertonic saline solution, as a part of medical treatment, to horses suffering from gastrointestinal colic, admitted to the Brazos Valley Equine Hospital, Navasota. Several circulatory and blood parameters were measured in an attempt to determine the effects of the administration of a bolus of HSS in comparison to the control group receiving a bolus of LRS. This study intends to reflect a practical approach to medical treatment of equine colic as seen on the day to day practice.

2.2. Materials and methods

2.2.1. Case enrolment criteria

Three criteria were set for a horse to enroll this study: suffer from gastrointestinal colic, be in need of IV fluid therapy and weight between 400 and 500 Kg.

Horses presenting with complaints of abdominal pain were assessed by one of the practitioners working at the hospital. A full physical exam was performed by the practitioner with help from one of the interns. Physical exam consisted of recording heart rate, capillary refill time, mucous membrane's color and moisture, jugular vein filling and rectal temperature. All horses were also subjected to an abdominal ultrasound, rectal palpation and to the passing of a nasogastric tube to confirm the presence of gastric reflux. Laboratory tests such as PCV, TS and plasma lactate were performed for all cases and more complete laboratory tests were also conducted if the practitioner decided so.

For some cases, the cause of colic was diagnosed after physical exam while for the rest it remained unknown. Horses for whom an exact diagnosis was not possible, but the possibility of a false colic was excluded, were considered to suffer from gastrointestinal colic.

Horses suffering from GI colic and that were in need of IV fluid therapy were admitted to the study. No criteria was defined for deciding if a horse should receive IV fluid therapy. The decision of administrating IV fluids relied solely on the practitioner in charge of the case, and it was based on the physical exam, lab results and cause of colic as happens on the day to day practice.

Since this study was intended to reflect a practical approach to the medical treatment of colics, it was defined that all horses enrolling in this trial were to receive a 2L bolus of fluid, as it is the common practice at BVEH for adult horses with colic to receive a fixed dose of 2L of hypertonic saline solution before receiving intravenous isotonic fluids. The initial criteria was for horses to weight between 400 and 500 Kg, which would correspond to a dose of 5 to 4 ml/Kg of HSS per

horse. However, due to the low caseload, three horses weighing slightly more than 500 Kg were admitted into the trial.

2.2.2. Blinding method

Both bottles of Lactated Ringer's Solution and Hypertonic Saline Solution were identical except for their label. The technician responsible for the hospital's pharmacy was asked to tape the labels of the bottles of both solutions, writing only "A" and "B" on each type (Figure 17). For the entire trial this technician was the only one to know which letter matched which fluid. A set of taped bottles was kept in the pharmacy, separated from the rest of the fluids, at all times, to avoid any kind of confusion.

Figure 17: Blinding method: 1L bottle of fluid with the label taped and the letter B written on it.

2.2.3. Randomizing method

Horses were randomized as to which type of fluid bolus they should receive.

Horses were grouped two by two and a coin was tossed for the first one of each group. To heads corresponded fluid A and tails matched fluid B. The second horse of the group would receive the type of fluid the first one didn't, allowing us to have a similar number of horses receiving boluses of HSS and control horses receiving LRS (HSS group and LRS group, respectively). Even though the horses enrolling this study were never admitted into the hospital at the same time, a tight record of the all the enrolled cases was kept, informing us to toss the coin or administer certain fluid to the horse being worked on at the time.

2.2.4. Administered drugs

All horses received a dose of flunixin meglumine calculated for their weight before being enrolled in the study. Three horses required sedation to facilitate handling and examination as well as a safety measure for the horse and staff. The administered sedation was of 200mg xylazine IV for each horse, which represented a dose of 0.40-0.44 mg/Kg. No effects of excessive sedation were noticed in any of the horses.

2.2.5. Parameter measurements

After a horse was enrolled in the study, a number of parameters were evaluated before, immediately after and 90 minutes (T0, T1 and T2, respectively) after the administration of the fluid bolus. These parameters consisted in measuring central venous pressure and non-invasive blood pressure as well as PCV, TS, plasma lactate and whole blood lactate, plasma biochemistry profile, and blood pH. All parameters were recorded on a data sheet created for each horse (Appendix 2). Due to the limitations concerning the length of this dissertation, a selection of the most relevant parameters for analysis and discussion was made. All blood

collection and sample handling operations, iSTAT® use and CVP and NIBP measurements were performed by the same person.

2.2.5.1 Administered fluids

As explained before, horses were randomized in groups receiving a 2L bolus of Vet One® 7,2% hypertonic saline solution and a control group that received a 2L bolus of Vet One® Lactated Ringer's Solution. As soon as all the necessary parameters for T0 were collected, the fluid boluses were administered to all horses through a 14 gauge IV catheter, placed in the jugular vein. After collecting the parameters for T1, all horses were put in a stall where, until T2, they received 10 litters of Plasma - Lyte A (Abbott Laboratories, North Chicago, IL) through their IV catheter. Horses were not allowed to drink water for the duration of the study. The composition of these fluids is described in Table 2 on chapter 1.2. After collection of the T2 parameters, all horses proceeded to receive the amount and type of fluids the responsible practitioner thought adequate.

Although commercial lactate analyzers are not supposed to detect the lactate present in the LRS, we noticed that the lactate analyzer used in this study detected a lactate concentration of 5,2mmol/L in the Vet One® Lactated Ringer's Solution used for the control group. These results will be presented and discussed later.

2.2.5.2. Central venous pressure and measurements

Central venous pressure was measured following the method described by Fielding et al. (2004). A sterile 1,2 mm x 56 cm, French Sovereign™ polypropylene catheter, filled with heparinized fluid, was inserted through a 14 gauge intravenous catheter previously placed on the jugular vein. Roughly one centimeter of the tip of the polypropylene catheter was cut off, previous to its insertion, in order to eliminate the blunt end and lateral openings. The catheter was then attached to an electronic pressure transducer connected to a Mindray™ PM-9000Vet monitor (Figure 18). The electronic pressure transducer was leveled with the point of the horse's shoulder tip and taped to it in order to maintain the same "zero" mark for all measurements, as described on chapter 1.6. Between T0 and T1 the pressure transducer was

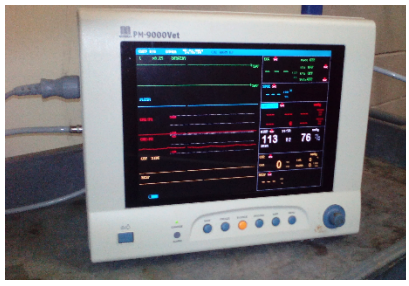


Figure 18: Mindray™ PM-9000Vet monitor used for measuring CVP and NIBP.

Figure 19: Pressure transducer taped to the horse's shoulder tip and a 20 mL syringe filled with heparinized fluid attached to the transducer. The bottom piece of tape was kept in the same place throughout all operations to avoid variations of the "zero" level.

kept attached to the horse. Between T1 and T2, since the horse would not stay in the stocks, the line of white tape attaching the pressure transducer was left in the horse's shoulder. A 20 mL syringe filled with heparinized fluid was kept attached to the pressure transducer and the entire line was flushed before and in between each CVP measurement (Figure 19).

Noninvasive arterial blood pressure was measured using the oscillometric sphygmomanometry method as described in chapter 1.7. by placing an inflatable cuff around the coccygeal artery on the base of the tail. The cuff was attached to the same monitor used for the CVP readings, which displayed both parameters simultaneously, and the horse was allowed to get used to the cuff inflation and deflation before NIBP values started being recorded. NIBP values were used as uncorrected values and the bladder width to tail girth ratio was not calculated, being that the hospital features a set of different sized inflatable cuffs.

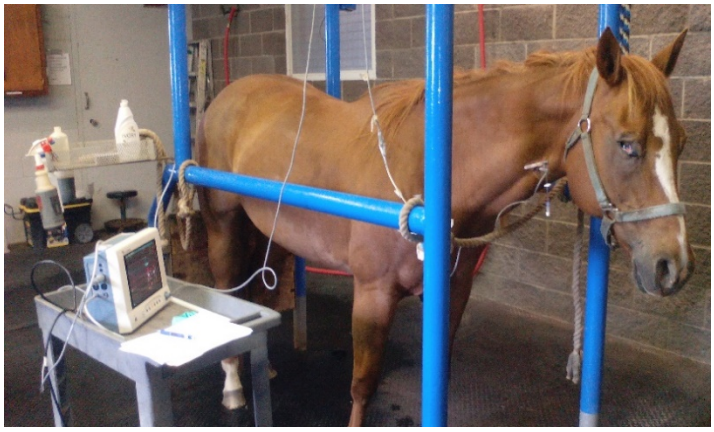


Figure 20: Mare in stocks while CVP and NIBP are being measured. Notice how she is minimally restrained and maintains her head in a nearly neutral position.

The horses were kept in stocks for all operations and efforts were made for the horse's head to stay in a neutral position with minimal restraining and for the animal to be relaxed and quiet (Figure 20). If the horse's behavior or position changed greatly the correspondent reading would be excluded. Three measurements were recorded for each parameter at each time point and the used value was the mean of these.

2.2.5.3. Packed cell volume, total solids, plasma lactate and plasma biochemistry

Blood was collected from the jugular vein of the side opposite to where the intravenous catheter was placed using a sterile 18 gauge needle and a 5 mL syringe. Immediately after collection, the blood was transferred into a plain blood tube under vacuum (red top) and a blood tube containing lithium heparin under vacuum (green top). The green top tube was then centrifuged for three minutes.

PCV was measured by filling two microhematocrit tubes and centrifuging them for three minutes. The PCV value was considered to be the mean of the two microhematocrit results unless there was a clear disparity among these in which case the technic would be repeated.

TS and plasma lactate were measured using plasma obtained from the centrifuged green top tube. TS were measured using a refractometer and plasma lactate was measured using a commercial point-of-care Nova Biomedical Lactate Plus® analyzer. This analyzer was found to have the lowest intra-analyzer variability and showed the best agreement to a bench top

analyzer, in blood of horses with colic, when compared to two other portable lactate analyzers (Nieto, Dechant, le Jeune, & Snyder, 2015).

A plasma biochemistry panel was analyzed using the bench top analyzer HESKA™ DRI-CHEM 7000. Although a full biochemistry panel was ran for each horse and each time point, we chose to present and discuss solely the values for albumin, blood urea nitrogen, creatinine, and total plasma protein due to their relation to prognosis in equine colic.

2.2.5.4. Venous blood pH and whole blood lactate

Blood pH and lactate was assessed using the commercial analyzer VetScan® iSTAT®1 and a CG4+ cartridge. The sample of blood used was collected into a blood tube under vacuum containing lithium heparin, as explained previously, but not centrifuged. Efforts were made to minimize the time between collecting the blood and placing the sample on the CG4+ cartridge. A new sterile syringe was used to transfer the blood sample from the blood tube to the cartridge and the first drops from the syringe were discarded. Even though the CG4+ cartridge provides information about blood gases and several other parameters, it was decided that blood pH and lactate would be the most relevant variables to discuss in this dissertation.

2.3. Statistical analysis

A descriptive statistics of our data was performed using the Rcmdr Version 2.3-0 software. Due to the small size of the sample, statistical differences were not calculated.

2.4. Results and discussion

A complete table of the obtained results is presented in the appendix section (Appendix 1).

2.4.1 Enrolled animals

Eight horses were admitted to this study resulting in an even distribution of four horses for the HSS group and for the LRS group. The description of the enrolled animals is presented on table 5.

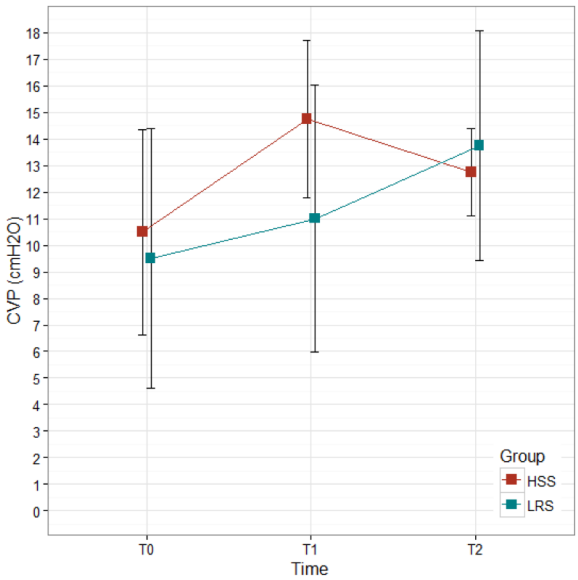
Table 5: Baseline characteristics of horses enrolled in this study receiving a 2L bolus of 7,2% hypertonic saline solution (HSS) or Lactated Ringer's Solution (LRS) (mean ± sd)

	HSS	LRS
N	4	4
Age (years)	11,25 ± 7,89	10,50 ± 3,87
Weight (Kg)	481,00 ± 38,50	483,75 ± 23,75
Breed	3 Quarter Horses 1 Gypsy Vanner	3 Quarter Horses 1 Thoroughbred
Gender	2 Geldings 2 Mares	1 Stallion 1Gelding 2 Mares
Diagnosis	1 Large colon displacement 1 Ileal impaction 1Colon impaction 1 Unknown	1 Large colon displacement 1 Ileal impaction 2 Unknown

2.4.2. Central venous pressure

CVP values for HSS and LRS groups at T0, T1 and T2 are represented in Graphic 1. CVP values for each horse at all time points are displayed on table 6.

Graphic 1: CVP values for HSS and LRS groups at T0, T1 and T2 (mean ± SE)



HSS	**1**	19	14	13
	2	3	9	9
	3	15	23	17
	4	5	13	12
LRS	**5**	3	5	6
	6	5	6	13
	7	24	26	26
	8	6	7	10

Table 6: CVP (cmH_2O) values for each horse at each time point.

Baseline CVP values were of 10,5 ± 7,72 cmH_2O for the HSS group and of 9,5 ± 9,75 cmH_2O for the LRS group, representing a high variability of these parameter's values among horses of both groups. Although both group's mean CVP increased after the administration of the fluid bolus, the LRS group's mean CVP increased less than 2 cmH_2O while the HSS group's increased in more than 4 cmH_2O. After 90 minutes (T2) the HSS's mean was lower than at T1 while the LRS's mean kept increasing. At T2 both group's means had converted to similar values at 12,75 ± 3,30 cmH_2O and 13,75 ± 8,66 cmH_2O for the HSS and LRS groups, respectively.

On the HSS group one of the horses (Horse 1 on Table 5) had a slight drop in CVP after receiving the fluid bolus while all other horses had visible increases. At first this was thought to be due to the peripheral vasodilation and consequent temporary hypotension as explained by Kien et al. (1998), Kreimeier & Messmer (2002) and Oliveira et al. (2002). However, this hypotensive period is usually shorter than the observed with this horse which lead us to believe the possibility of a technical error having happened in T0 that caused a falsely high CVP value, such as a kinked catheter tip, air within the lines or some kind of catheter obstruction (Fielding et al., 2004; Magdesian, 2004).

There was one mare on the LRS group (Horse 7 on Table 5) whose T0 CVP value was of 24 cmH_2O and had risen to 26 cmH_2O at T1 and T2. The diagnose was a displaced large colon and the mare was presented with a distended left flank, being that the abnormally high CVP was attributed to the increase in intraabdominal pressure (Gelman, 2008; Magdesian, 2004). However, less than 24 hours later, the mare was no longer distended or painful but the CVP remained equally elevated. No cardiac, pulmonary or other kind of cause was found to justify these values

Never the less, and even though the number of horses was too low to allow us to conclude about any kind of significance, the behavior of the values observed on the graphic matches the expected, as the plasma expansion and increased cardiac function caused by the administration of a hypertonic solution will lead to an increase in intravascular volume and, therefore, of the central venous pressure (Schmall, 1989; Schmall et al., 1990; Pantaleon, 2005; Taylor & Clark, 2007; Robertson, 2010; Theobaldo et al., 2012; Magdesian, 2015b). However this effect is of short duration, explaining the lower values at T2 compared to T1 for the HSS group. The values for the LRS group also behave as expected since all horses received a 10L bolus of isotonic solution between T1 and T2, explaining the improvement in this group's CVP mean.

Although baseline CVP measurements may prove to be useful, especially in the presence of extreme values, a lot of patients may be under serious hemodynamic derangements and still

have a central venous pressure within normal limits. This is due to the body's responses towards maintaining homeostasis, which enhances the importance of performing continuous or serial CVP measurements as a way to monitor the therapy's effects and the patient's condition (Gelman, 2008; Magdesian, 2015b). All horses, except for Horse 1 mentioned above, had higher CVP values at T2 than at admission (T0). This fact lead us to believe that even the animals who presented baseline CVP values considered within normal range were probably in need for some kind of fluid replacement.

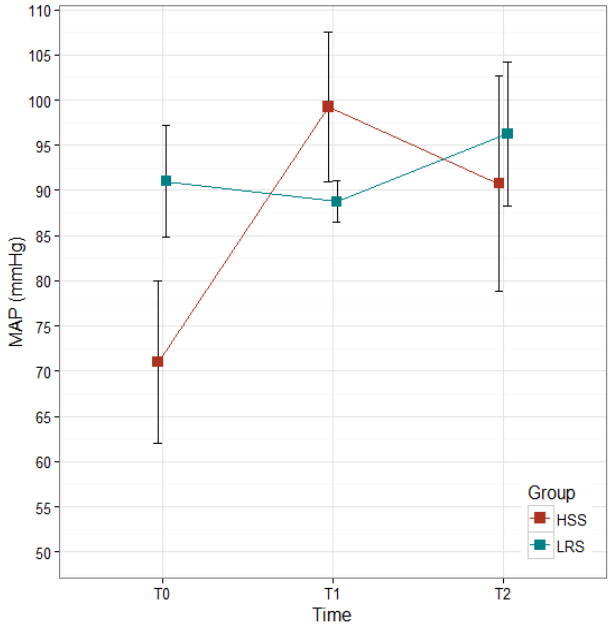
2.4.3. Noninvasive blood pressure

Mean arterial pressure values for the HSS and LRS groups at each time point are represented on Graphic 2. MAP values for each horse at each time point are presented on Table 7.

Table 7: MAP values (mmHg) for each horse at each time points.

Group	Horse	T0	T1	T2
HSS	**1**	70	89	82
	2	49	123	112
	3	93	98	108
	4	72	87	61
LRS	**5**	79	88	78
	6	86	95	111
	7	91	84	88
	8	108	88	108

Graphic 2: Noninvasive MAP mean ± SE for HSS and LRS groups at T0, T1 and T2.



As explained on chapter 1.7, a minimal MAP of 50 to 60 mm Hg is fundamental for an adequate organ perfusion and it's this value, more than systolic and diastolic arterial pressures that secures perfusion in the critically ill patient (Magdesian, 2004).

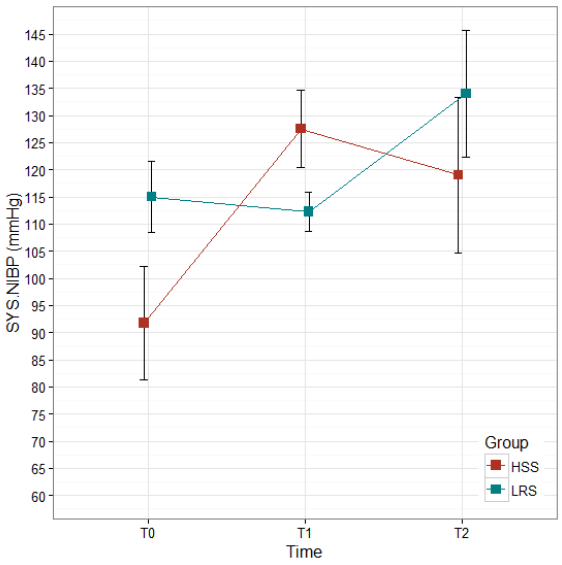
Even though the mean baseline of the HSS group (71 ± 17,98) was considerably lower than the LRS group's (91 ± 12,36), at T1 it had risen to values above the LRS ones, returning to lower values, but still higher than at T0, at T2. The mean MAP for the LRS group decreased slightly at T1 but after 90 minutes it showed some improvements as expected after the administration of 10L of Plasma – Lyte A.

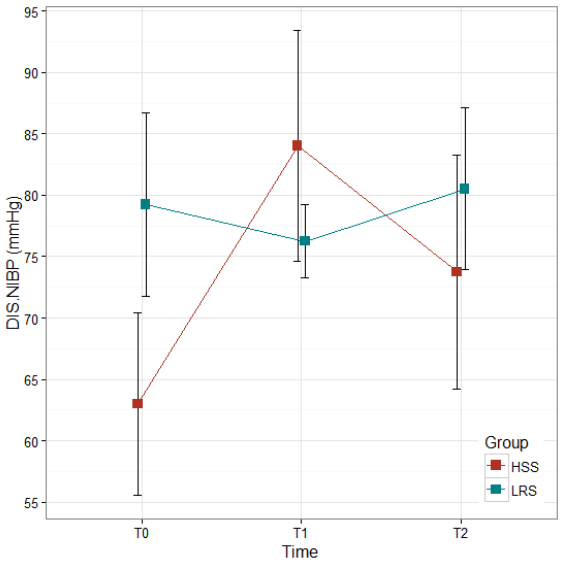
Only one horse of each group (Horse 4 and 7 on Table 7) had lower MAP values at T2 than at T0. Although the decrease in MAP for Horse 7 doesn't seem to be relevant, the decrease in

MAP for Horse 4 may be attributed to a higher stress and/or pain level at T0, to the worsening of the patient's circulatory status despite the administered fluids or other possibilities described on chapter 1.7 such as the onset of endotoxemia or acid-base derangements (Gay et al., 1977).

Horse 2 on Table 7 exemplifies a good situation where a horse benefits from the administration of HSS. This horse was clearly hypovolemic (see also Table 6, Horse 2) and the administration of a HSS bolus secured appropriate central venous pressure and mean arterial pressure until a larger volume of isotonic fluids was administered.

Graphic 3 and Graphic 4: noninvasive systolic (SYS.NIBP) and diastolic (DIS.NIBP) arterial pressures mean ± SE for HSS and LRS groups at T0, T1 and T2





Gay et al. (1977) showed how low systolic blood pressure was associated to a poor prognosis in horses undergoing colic surgery and later, Parry, Anderson, and Gay (1983), corroborated the same by reporting how low systolic and diastolic arterial pressures were related to mortality in horses with colic.

In our study, mean systolic and diastolic arterial pressures behaved in a similar pattern as MAP for both groups as can be observed on Graphics 3 and 4. Once more the HSS group's values behaved as expected, increasing after the HSS bolus caused a plasma expansion and improved the cardiac function, and decreasing at T2 due to the short duration of the HSS effects but still maintaining the effects of the 10L bolus and maybe even some remaining effects of the hypertonic solution.

The decreases of all three parameter's mean between T0 and T1 for the LRS group may be explained by one horse (Horse 8 on Table 7) whose MAP, systolic and diastolic arterial pressures decreased abruptly between T0 and T1, impacting the group's means. This decrease was attributed to the reduction of the initial stress as the horse overcame stressful stimulus like transport, handling and physical exam.

Errors concerning technic execution are also a possibility that can't be overlooked. Even though the bladder width to tail girth ratio was not calculated, the same inflatable cuff was used for each horse across all time points and, since the hospital owns a set of different sized cuffs, it was possible to select the most appropriate one for each horse. There was no correction of the obtained results but the goal of this study was to observe the variations of arterial blood pressure at different time points rather than its exact values.

2.4.4. Packed cell volume

Mean PCV values for both groups at each time point are represented on Graphic 5. PCV values for each horse at each time point are presented on Table 8.

Mean baseline PCV values were similar for both groups (45 ± 8,72 % for the HSS group and 45,25 ± 4,57% for the LRS group), yet the mean hematocrit for the HSS group suffered a bigger drop from T0 to T1 than the LRS group's one.

Graphic 5: PCV mean ± SE for HSS and LRS groups at T0, T1 and T2.

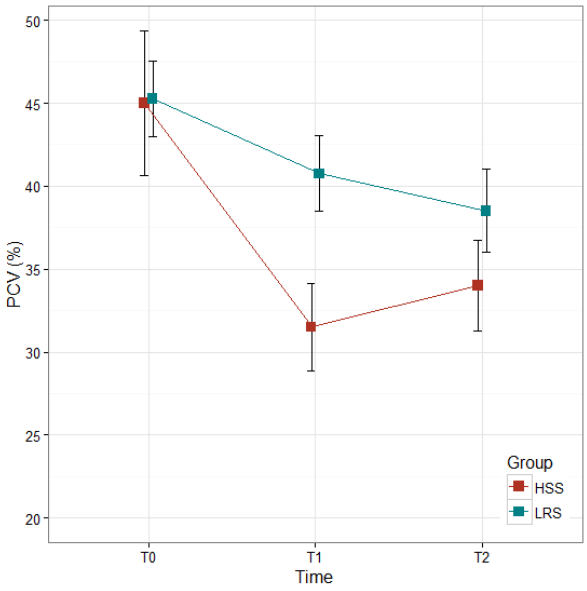


Table 8: PCV values (%) for each horse at each time points.

Group	Horse	T0	T1	T2
HSS	**1**	50	36	36
	2	48	32	36
	3	32	24	26
	4	50	34	38
LRS	**5**	43	43	40
	6	50	44	44
	7	40	34	32
	8	48	42	38

Even though the size of the sample is too small to allow us to calculate the existence of a significant difference between both groups, by observing Table 8 it's possible to conclude that the drop in PCV, between T0 and T1, for the HSS group ranged from 8 to 16%, with a mean drop of 13,5%, while for the LRS group it was of 0 to 6%, with a mean 4,5%. Even at T2, after all horses received a 10L bolus of isotonic fluids, the drop in PCV, compared to baseline, for the HSS group ranged from 6 to 14%, with a mean of 11%, while for the LRS group it went from 3 to 10%, with a mean drop of 6,75%. A bigger drop in PCV in the HSS group compared to the LRS group is consistent with previous studies conducted in this field (Schmall et al., 1990; Bertone & Shoemaker, 1992; Fielding & Magdesian, 2011).

The hematocrit may be pathologically elevated in situations of acute blood loss or hypovolemia (Corley, 2008a; Ethell et al., 2000; Nolen-Waltson et al., 2011; Fielding & Magdesian, 2015). Knowing that a PCV greater than 45% usually means there's some contraction of the plasma volume (Ethell et al., 2000) and that hematocrits above 50% are almost always present in

hypovolemic horses (Corley, 2008a), we can conclude that at least five of the enrolled horses had some degree of plasma volume deficit.

However, PCV values are extremely variable among breeds and even among individuals within the same breed. Horse 3 was a Gypsy Vanner, a breed of draft horse. According to Sellon (2010), PCV values for draft horses range from 24 to 44%, meaning that, even though this horse's hematocrit seemed normal at first, it could, in fact, be elevated for that individual.

Besides differences among individuals, PCV can be physiologically elevated by splenic contractions in response to stress or excitement ((Rose & Hodgson, 2000; Sellon, 2004; Corley, 2008a; Nolen-Waltson et al., 2011)). Horses from our study were painful, submitted to, sometimes long, trailer rides and to relatively invasive physical exams (rectal exam, nasogastric intubation, etc.) which could all contribute for splenic contraction and increases in PCV.

All the factors capable of influencing the PCV decrease this test's liability, especially when used individually. Ideally, PCV should be interpreted along with total solids or total plasma protein, at least, and measured serially to monitor therapy.

2.4.5. Total solids, total protein and albumin

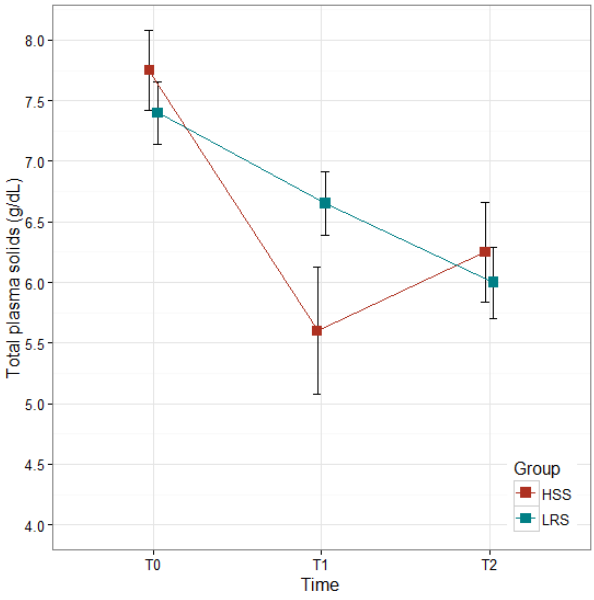
Mean total solids and total plasma protein values for both groups at each time point are represented on Graphics 6 and 7, respectively.

The concentrations of TS are similar to the concentration of TP at all time points which indicates us that there was little or no interference of the remaining plasma components with the refractometer's reading (Weiser, 2012). However, for one horse of each group the TP at T0 were 0,5 g/dL lower than the TS and for one horse of the LRS group the baseline TP was 0,9 g/dL lower than the TS. These differences are hard to explain being that they could be due to technical errors or the presence of plasma constituents that affected the refractometer's reading.

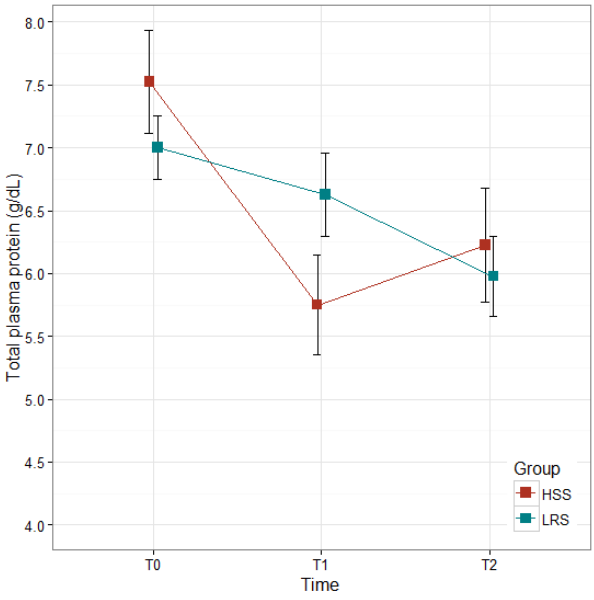
At T1 and T2 the maximum difference between TS and TP was of 0,4 g/dL.

The concentration of plasma protein is an important tool in assessing volemia status as long as ongoing or recent protein losses are excluded (Ethell et al., 2000; Magdesian, 2015a). None of the horses enrolled in our study presented, or developed in the following 24 hours, any signs of protein losses like diarrhea or nephropathy, leading us to consider the obtained TS and TP as reliable hypovolemia indicators.

Graphic 6: Total plasma solids mean ± SE for HSS and LRS groups at T0, T1 and T2.



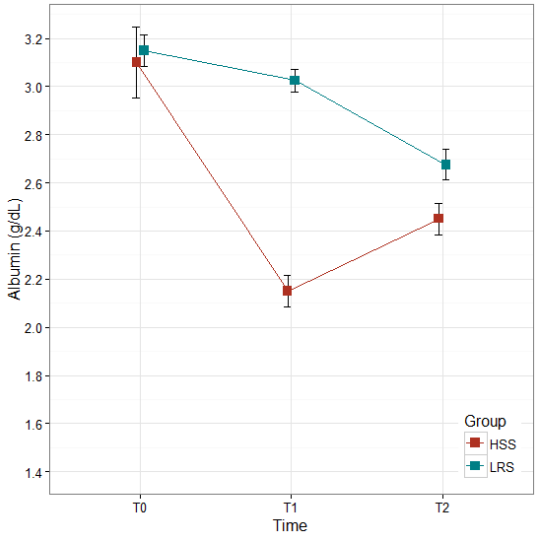
Graphic 7: Total plasma protein mean ± SE for both HSS and LRS groups at T0, T1 and T2.



Orsini and Divers (2014) consider hypovolemia according to TP as mild for 7 - 8 g/dL, moderate for 8 - 9 g/dL and severe for TP values above 9 g/dL. Rose and Hodgson (2000) consider that horses with TP values under 7,5 g/dL don't require fluid therapy while animals with TP values ranging from 7,5 to 8,5 g/dL have indication for IV fluids and horses with plasma protein concentrations greater than 8,5 g/dL definitely require intravenous fluids.

Mean baseline TP for the HSS and LRS groups were 7,53 ± 0,82 g/dL and 7,0 ± 0,5 g/dL, respectively, and only one horse of each group had a TP concentration inferior to 7 g/dL.

Graphic 8: Albumin mean ± SE for the HSS and LRS groups at T0, T1 and T2.



Graphic 8 represents the mean Albumin values for both groups at all time points. As described above none of the enrolled horses presented, or developed in the following 24 hour period, signs that indicated possible albumin losses.

Normal albumin concentrations for horses range from 2,3 to 3,8 g/dL (Corley & Stephen, 2008; Orsini & Divers, 2014). Hyperalbuminemia in horses only seems to occur in severe dehydration (Corley & Stephen, 2008) and none of the horses of our study presented albumin concentrations out of the normal range.

Even though the study's sample is too small to allow us to calculate the presence of significant differences between groups, by observing the Graphics 6, 7 and 8 it's possible to notice that the drop in TS, TP and Albumin between T0 and T1 is greater for the HSS group than for the LRS group. This decrease in TP is consistent with previous studies conducted in this field (Schmall et al., 1990; Bertone & Shoemaker, 1992; Fielding & Magdesian, 2011). Between T1 and T2, total protein, total solids and albumin values for the HSS group increased as the effects of the hypertonic saline solution bolus wear off, however they remained inferior to T0 as the 10L bolus of isotonic fluids maintained some of the plasma volume expansion. The LRS group's values for the same variables decreased along the two time intervals (T0-T1 and T1-T2) as 2 and 10 litters of isotonic fluids were added to the plasma volume.

Plasma protein concentrations of less than 4 g/dL and albumin concentrations inferior to 1 - 1,5 g/dL can be considered as the limit for edema formation in horses (C. L. Fielding, 2015). None of the horses presented, at any time point, TP or albumin values inferior to these, which lead us to believe that there was no need to add or replace the crystalloid fluids by synthetic or natural colloids.

2.4.6. Creatinine and BUN

Mean creatinine and BUN values for both groups at each time point are represented on Graphics 9 and 10 respectively.

Mean baseline creatinine for the HSS and LRS groups were 2,02 ± 0,33 mg/dL and 1,45 ± 0,52 mg/dL, respectively.

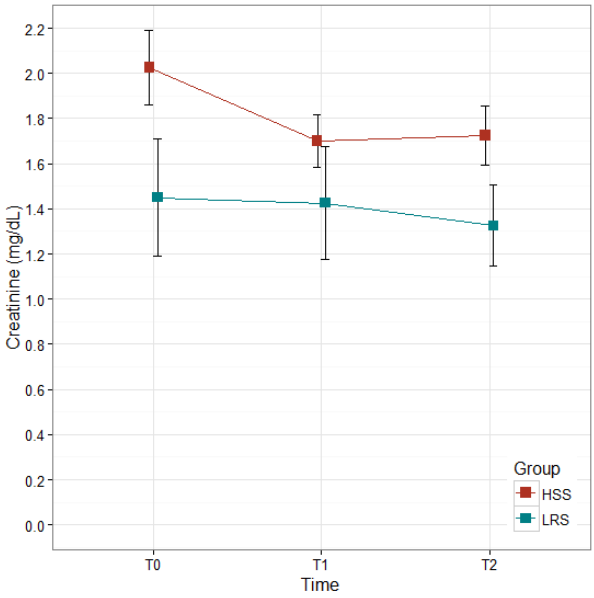
Normal serum creatinine concentrations for adult horses range from 0,9 to 2 mg/dL (Orsini & Divers, 2014; Fielding, 2015b) although it has been reported that normal adult Quarter horses and Warmbloods may have creatinine levels up to 2,4 mg/dL (Orsini & Divers, 2014; Radcliffe et al., 2015).

Even though all of the horses of our study had creatinine concentrations within the normal range, high normal values could indicate the presence of subclinical hypovolemia (Corley, 2008a).

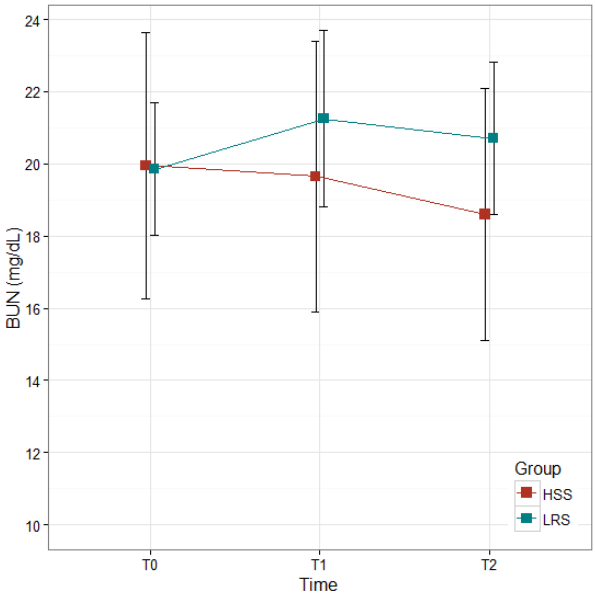
Normal levels of blood urea nitrogen in adult horses range from 10 to 27 mg/dL according to Hollis (2008) and Orsini and Divers (2014)

Mean baseline BUN values for the HSS and LRS groups were 19,95 ± 7,35 mg/dL and 19,85 ± 3,67 mg/dL, respectively.

Graphic 9: Creatinine mean ± SE for the HSS and LRS groups at T0, T1 and T2.



Graphic 10: BUN mean ± SE for the HSS and LRS groups at T0, T1 and T2.



Gastrointestinal diseases are one of the most common causes for kidney injury due to hypoperfusion (Radcliffe et al., 2015; Schott II, 2010) and, in horses suffering from abdominal colic, higher blood concentrations of creatinine and urea proved to be related with increased mortality (Buchanan, 2014).

Creatinine concentrations for the LRS group remained approximately the same between T0 and T1 and decreased slightly at T2.

In our study, the HSS group showed a slight reduction in creatinine levels at T1, however, the same did not happen for the BUN levels, which remained practically the same after the 2L bolus of HSS.

In opposition to what happened with the previous blood parameters, there is a slight increase in BUN after the administration of the two litter bolus of lactated Ringer's solution. No relation between the administration of LRS and increases in urea production was found, however, it has been proposed that increased oxygenation of liver tissue promoted the production of urea whereas liver hypoxia reduced this molecule's synthesis (Opie, 1960, 1961). Even though the administered volume of LRS was of only two litters, it is possible that this amount of fluids was enough to increase liver perfusion and urea production. The administration of HSS was also likely to increase liver perfusion and, consequently, cause an increase in total BUN, nevertheless, because of the simultaneous plasma expansion, BUN concentration at T1 remained similar to T0.

At T2, BUN concentrations decreased slightly in both groups, most likely due to the dilutional effect of the 10L bolus of Plasma - Lyte A as well as increased renal excretion.

The administration of hypertonic saline solution showed to increase blood flow to the kidneys (Schmall, 1989; Kien et al., 1998). The improved kidney perfusion and higher plasma sodium concentration after HSS administration result in a shorter time to urination, increased urinary frequency and more diluted urine, which is believed to act as a protective effect against renal failure associated with hypoperfusion (Fielding & Magdesian, 2011).

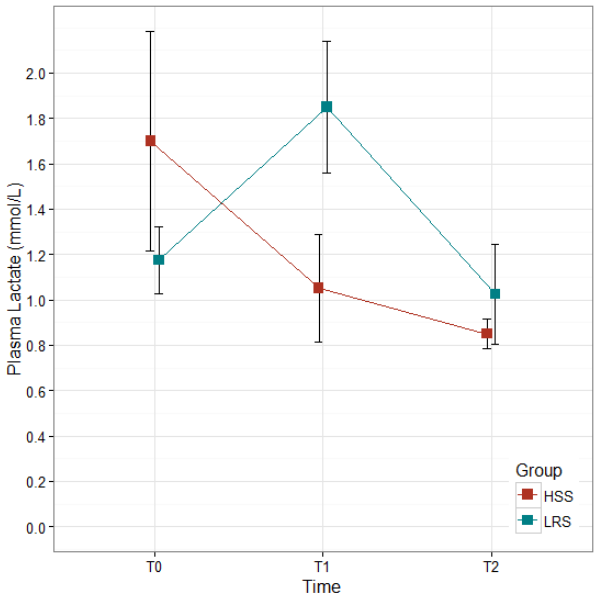
2.4.7. Plasma lactate and blood lactate

Mean plasma and whole blood lactate values for both groups at each time point are represented on Graphics 11 and 12, respectively.

Both lactate values were obtained using two different commercial analyzers as explained on chapter 2.2.5.4. Whole blood lactate measurements were consistently lower than plasma lactate, probably due to the dilutional effect of the erythrocytes, which are poorer in lactate than plasma (Radostits et al., 2007).

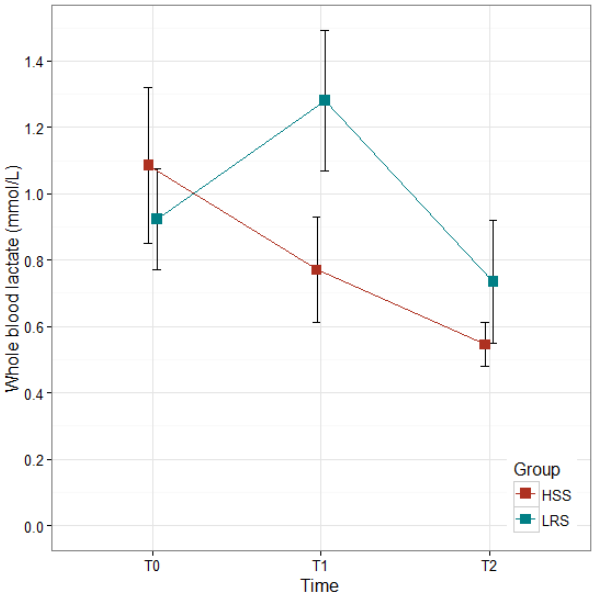
Despite de differences in values, both plasma measurements behave in a similar manner along the time points.

Graphic 11: Plasma lactate mean ± SE for the HSS and LRS groups at T0, T1 and T2.



The increase in lactate concentration after the administration of the LRS bolus (T1) can be explained by the ability of the analyzers to detect this solution's lactate. Although it was not tested on the iStat®, we tested the Vet One® Lactated Ringer's Solution on the Nova Biomedical Lactate Plus® which detected 5,2 mmol/L of lactate present in this solution. This means that for each 2L bolus of LRS the horses received 10,4 mmol of lactate detectable by our analyzer. If we consider the plasma volume to be 0.052 to 0.063 L/kg in healthy adult horses (Fielding, 2015a), for a 450 Kg this would result in an increase of 0,37 to 0,44 mmol/L in plasma lactate. It is important to mention that this calculation does not take in account several variables such as the metabolization rate of such lactate and the volemia status of the horse at that time.

Graphic 12: Whole blood lactate mean ± SE for the HSS and LRS groups at T0, T1 and T2.



Nonetheless, the animals from the HSS not only experienced a considerable drop in lactate at T1 as these values remained lower than those of the LRS group at T2, regardless of the initial group mean being higher than the LRS group's.

Normal plasma lactate values for adult horses are generally considered to be less than 1,5 mmol/L (Nappert & Johnson, 2001; Fielding & Magdesian, 2005;) or 2,0 mmol/L (Magdesian, 2004), and increases in blood lactate concentrations have been strongly related to worse prognosis in several species as explained on chapter 1.9.

Even though the plasma lactate levels of the horses enrolled in our study were not alarmingly high, the administration of hypertonic saline solution was likely to improve microhemodynamics and organ perfusion. This effect justifies how lactate concentrations for the HSS group kept diminishing, whereas the other blood variables increased from T1 to T2, after the effects of the hypertonic solution ended. The continuous decrease in lactate, can't be attributed solely to the dilution of the bloods constituents after plasma expansion but also to the decline in lactate production by the cells upon circulation improvements.

2.4.8. Blood pH

pH mean for both groups at T0, T1 and T2 are presented in Graphic 13.

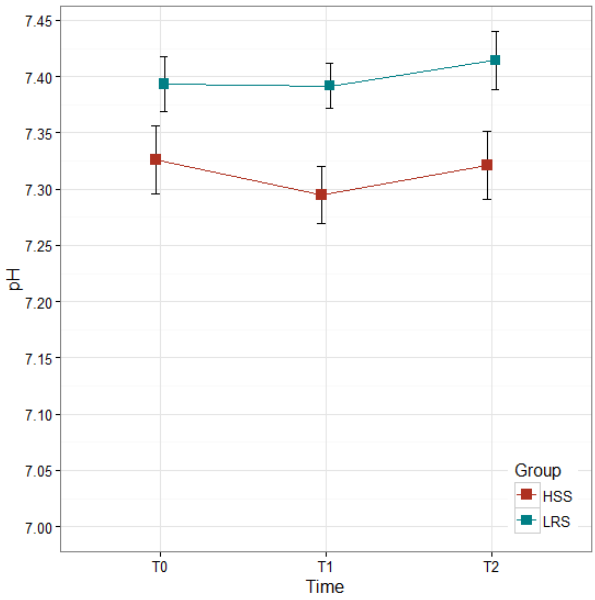
Although blood pH was not extensively reviewed in this dissertation, there is the eventual concern about causing an iatrogenic acidosis by the administration of hypertonic saline due to its low pH and absence of a buffer component.

Mean pH at T0 and at T1 for the HSS group was of 7,33 ± 0,06 and 7,30 ± 0,05, respectively, which represents a decrease of 0,03 units in mean pH between T0 and T1 for the HSS group.

The biggest decrease in pH for the HSS group was of 0,045 units which fits Constable's (2003) statement that the decrease in pH caused by HSS in large animals is less than 0.08.

At T2 the mean pH for the HSS group was similar to T0, indicating that, besides being of little value, the drop in pH is also short lasting in animals receiving hypertonic saline solution.

Graphic 13: pH mean ± SE for the HSS and LRS groups at T0, T1 and T2.



2.5. Study limitations and future considerations

This was a preliminary study and it is our intention to gather data from a larger sample in order to achieve a greater statistical power.

The biggest limitation of our study was the sample size. However, we were limited by the enrollment criteria created in the beginning. Although more than eight horses fit the enrollment criteria, some owners declined IV fluid therapy due to budget limitations.

The heterogeneity of the group of horses used was another limitation for our study. Among this sample there were horses of different ages, weights, diagnosis and breeds. It was also hard to define the time that passed between the onset of colic and the hospital admission for these horses. Nonetheless, we hope that increasing our sample will balance both groups and reflect a larger population.

All horses had at least one variable that could be considered as being within normal limits, which could lead to the debate about the need for some animals to receive IV fluids. However, the goal of this study was to reflect a practical approach to colic treatment and it was decided that it would be up to the practitioner in charge of each case to decide about the horse's need for VI fluid therapy. A larger sample is expected to reflect the hemodynamic status of horses with medical colics with greater accuracy.

The administration of a bolus of LRS proved to interfere with the real plasma lactate concentrations, and probably the BUN concentrations as well, of the LRS group. This should be taken into account and the replacement of the LRS by a lactate free isotonic polyionic solution should be considered before proceeding with more data collection.

Due to the length limitations of this dissertation more variables were collected than the ones presented and discussed here. However it is our intention to present these variables in the future and after the gathering of a larger, more representative sample.

Even though these were not the goals of this work, it could be interesting to further investigate the amount of fluids each horse received before being released from the hospital, the effects of HSS in specific types of colic, the reason for the increases in BUN after the administration of LRS and if the same is true for the administration of HSS.

Conclusion

The major limitation of this work was the small sample size, which didn't allow us to calculate the existence of significant differences between horses treated with hypertonic saline solution and the control group. However, the pattern observed for most variables of the HSS is clear.

Central venous pressure and arterial blood pressures increased visibly after treatment with HSS, dropping after the effect of this solution had passed. However, at the end of 90 minutes and after the administration of 10 litters of isotonic crystalloids, CVP and arterial blood pressures were still higher than baseline.

The opposite happened for blood parameters known to be related with worse prognosis in equine colic, such as PCV, TP and creatinine, that decreased after treatment with HSS and increased slightly after the effects of the HSS worn off and the 10 litter bolus of isotonic fluids was administered.

Plasma lactate decreased continuously after the administration of the HSS bolus and of the 10L isotonic fluid, indicating us that, even after the effects of the hypertonic solution had passed, organ perfusion was improved and lactate production by the body's cells had stopped.

The effects of HSS like the increase in plasma volume and cardiac function, the reestablishment of microhemodynamics and improvement of organ perfusion, and the reduction of tissue lesions due to ischemia or inflammatory processes are believed to exert protective effects even in horses with subclinical hypovolemia.

A single bolus of HSS seems to promote a bigger increase in blood flow to organs like heart, kidney, liver and intestines, which allows us to quickly secure vital functions while administering larger volumes of isotonic crystalloids.

Despite the small size of our sample, it is possible to affirm that some of the effects obtained at T1 for the HSS group were only achieved at T2 for the LRS group.

Although the dose of HSS administered to some horses was superior to the standard 4 ml/Kg of body weight, no deleterious effects were noticed with doses up to 4,6 ml/Kg.

The observed results support our hypothesis that horses with medical colics also benefit from treatment with HSS and encourage us to proceed with data collection in order to obtain a larger sample and stronger statistical results.

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Appendix 1 – Results table

		HSS vs LRS groups values according to time point											
		T0				T1				T2			
		$\bar{x}$	SD	SE	CI	$\bar{x}$	SD	SE	CI	$\bar{x}$	SD	SE	CI
PCV (%)	HSS	45,00	8,72	4,36	13,87	31,5	5,26	2,63	8,37	34,0	5,42	2,71	8,62
	LRS	45,25	4,57	2,29	7,28	40,75	4,57	2,29	7,28	38,50	5,00	2,50	7,90
ALB (g/dL)	HSS	3,10	0,29	0,15	0,47	2,15	0,13	0,06	0,21	2,45	0,13	0,06	0,21
	LRS	3,15	0,13	0,06	0,21	3,02	0,10	0,05	0,15	2,68	0,13	0,06	0,20
TS (g/dL)	HSS	7,75	0,66	0,33	1,05	5,85	0,82	0,41	1,31	6,25	0,82	0,41	1,31
	LRS	7,40	0,52	0,26	0,82	6,65	0,53	0,26	0,84	6,00	0,59	0,29	0,94
TP (g/dL)	HSS	7,53	0,82	0,41	1,30	5,75	0,79	0,40	1,26	6,23	0,91	0,46	1,44
	LRS	7,00	0,50	0,25	0,80	6,63	0,67	0,33	1,06	5,98	0,63	0,32	1,01
BUN (mg/dL)	HSS	19.95	7,35	3,67	11,69	19,65	7,50	3,75	11,94	18,60	6,98	3,48	11,0
	LRS	19,85	3,67	1,84	5,84	21,25	4,89	2,45	7,79	20,70	4,21	2,10	6,69
CRE (md/dL)	HSS	2,02	0,33	0,17	0,53	1,7	0,23	0,12	0,37	1,73	0,26	0,13	0,42
	LRS	1,45	0,52	0,26	0,83	1,43	0,50	0,25	0,79	1,33	0,36	1,18	0,57
CVP (cmH_2O)	HSS	10,5	7,72	3,86	12,29	14,75	5,91	2,95	9,40	12,75	3,30	1,65	5,26
	LRS	9,50	9,75	4,87	15,51	11,00	10,03	5,02	15,97	13,75	8,66	4,33	13,7
MAP (mmHg)	HSS	71,0	17,98	8,99	28,61	99,25	16,54	8,27	26,32	90,75	23,88	11,94	38,0
	LRS	91,00	12,36	6,18	19,66	88,75	4,57	2,29	7,28	96,25	15,88	7,94	25,2
SYS (mmHg)	HSS	91,75	20,89	10,44	33,24	127,50	14,15	7,08	22,52	119,0	28,5	14,25	45,3
	LRS	115,00	13,09	6,54	20,83	112,25	7,27	3,64	11,58	134,0	23,34	11,67	37,1
DIS (mmHg)	HSS	63,0	14,90	7,45	23,71	84,0	18,83	9,42	29,97	73,75	19,09	9,54	30,3
	LRS	79,25	14,93	7,47	23,76	76,25	5,91	2,95	9,40	80,50	13,18	6,59	20,9

PCV: packed cell volume; ALB: albumin; TS: total solids; TP: total protein; BUN: blood urea nitrogen; CRE: creatinine; CVP: central venous pressure; MAP: mean arterial pressure; SYS: systolic blo
pressure; DIS: diastolic blood pressure

		HSS vs LRS groups values according to time point (cont.)											
		T0				T1				T2			
		$\bar{x}$	SD	SE	CI	$\bar{x}$	SD	SE	CI	$\bar{x}$	SD	SE	CI
PLac (mmol/L)	HSS	1,70	0,97	0,48	1,54	1,05	0,47	0,24	0,75	0,85	0,13	0,06	0,21
	LRS	1,18	0,30	0,15	0,48	1,85	0,58	0,29	0,92	1,03	0,44	0,22	0,70
BLac (mmol/L)	HSS	1,09	0,47	0,24	0,75	0,77	0,32	0,16	0,50	0,55	0,13	0,07	0,21
	LRS	0,92	0,31	0,15	0,49	1,28	0,42	0,21	0,67	0,74	0,37	0,19	0,59
pH	HSS	7,33	0,06	0,03	0,10	7,30	0,05	0,03	0,08	7,32	0,06	0,03	0,1
	LRS	7,39	0,05	0,02	0,08	7,40	0,04	0,02	0,06	7,41	0,05	0,03	0,08

PLac: plasma lactate; BLac: whole blood lactate

Appendix 2 – Data sheet

Hypertonic Saline Solution

A **B**

Horse:	**Date:**	**Diagnosis:**
Age:	**Gender:**	**Breed:**

Weight	**0, 1 & 90 min:** Chemistry + Electrolytes + CG4
Kg 0:	
Kg 24h:	**0 & 24h:** WBC

Physical exam

HR 0:	JF 0:	CRT 0:
HR 1:	JF 1:	CRT 1:
HR 90:	JF 90:	CRT 90:

Blood pressure (3 measurements each)

NIBP 0:	MAP 0:	CVP 0:
NIBP 1:	MAP 1:	CVP 1:
NIBP 90:	MAP 90:	CVP 90:

Lab work

LAC 0:	TS 0:	PCV 0:
LAC 1:	TS 1:	PCV 1:
LAC 90:	TS 90:	PCV 90:

Notes: